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STUDIES ON THE ESTIMATION OF CARDIAC OUPTUT IN MAN, AND OF ABNORMALITIES IN CARDIAC FUNCTION, FROM THE HEART'S RECOIL AND THE BLOOD'S IMPACTS; THE BALLISTOCARDIOGRAM

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The movements imparted to the human body by the impacts of the blood were first recorded by Yandell Henderson (1905) by means of a "swinging table" and a set of levers. His subjects had to arrest or suppress respiration before a record of movements synchronous with the heart beat could be obtained. A relation between cardiac output and the amplitude of these movements was suggested. On the Pike's Peak expedition (Douglas et al., 1913) a simplified form of the apparatus, a plank supported on piles of corks, was used to ascertain whether altitude affected cardiac output.

Heald and Tucker's (1922) subject stood on a platform suspended from the diaphragm of a drum. Movement was recorded by changes in the current flowing through a hot wire placed in the drum's air inlet. The direction of the movements could not be ascertained from their records. Nevertheless normal respiratory activity did not interfere with the record of the cardiac movements and the effect of the respiratory cycle on these movements was clearly shown. Increased amplitude was demonstrated after exercise, after nitroglycerine, and in a patient with aortic regurgitation.

Angenheister and Laue (1928) published 5 records obtained from a seismograph placed on a rigid table on which their subject lay. Four of these records are similar to those we obtain.

Abramson (1933) built a chair of aluminum alloy suspended from steel springs which were embedded in a cast steel base on a concrete foundation. The movements were greatly magnified by a patented device and photographed. The great nicety of construction was indicated by the high vi-

bration frequency, 75 per second with 70 kgm. of dead weight on the chair. The records from Abramson's "Kardiodynamograph" were essentially similar to those we obtain.

From theoretical conceptions Abramson (1933) developed a formula relating the double integral of his curves to the cardiac output. Experiments on this subject were projected but we can find no record of the results obtained. Our experiments indicate that cardiac output cannot be estimated from this formula.

Our apparatus is a modification of the hanging table originally employed by Henderson (1904) and so is much simpler and less expensive than Abramson's (1933) apparatus. Instead of a table swinging free, which we tried and abandoned, we have opposed the longitudinal motion by a strong steel spring, a change which has abolished the interference due to the movements of normal respiration. Optical methods of magnification and recording have supplanted the original lever and kymograph system.

With this apparatus we have confirmed the findings of our predecessors in this field regarding the general shape of the normal cardiac ballistic curve and the effect of exercise, and certain drugs, upon its amplitude. We have also conducted extensive experiments into the sources of error, and found that these are due to certain properties of the human body rather than to defects of apparatus. The knowledge gained has suggested methods of avoiding or minimizing these errors. With this as a background we have developed new theoretical conceptions of the forces involved and have derived new formulae for estimating cardiac output. The great majority of results obtained from these formulae agree well with those secured by the improved ethyl iodide method (Starr and Gamble, 1927).

The design and construction of our apparatus was the work of Rawson. Joseph performed the analyses of ethyl iodide and assisted in the estimation of cardiac output by that method. We received valuable assistance from Dr. H. K. Hartline in setting up the optical recording system, from Dr. Alan Burton in certain physical and mathematical aspects of our problems, and from Dr. Alexander Margolies in recording the heart sounds in two experiments. Dr. C. C. Wolferth loaned us an electrocardiograph, Dr. H. C. Bazett gave helpful advice about numerous matters, and Dr. C. J. Wiggers made a suggestion which we promptly investigated.

APPARATUS. The table, figure 1, is constructed of a thin 3 ply panel mounted on a spruce frame. To stop vertical vibrations of the central part of the table it was found necessary to brace it by the steel truss shown. The total weight is approximately 50 pounds.

Lateral motion of the table is prevented by two struts each of which is attached both to the wall and to the table through a flexible joint so that they offer no resistance to longitudinal motion.

The adjustable spring, a piece of hardened tool steel $\frac{1}{8}$ x $\frac{3}{4}$ inch, is shown

in detail in figure 1. It is mounted on a rigid welded steel structure, of pipe and channel, screwed to the floor and braced against the side wall. The mirror is mounted on a very short lever of phosphor bronze, one end

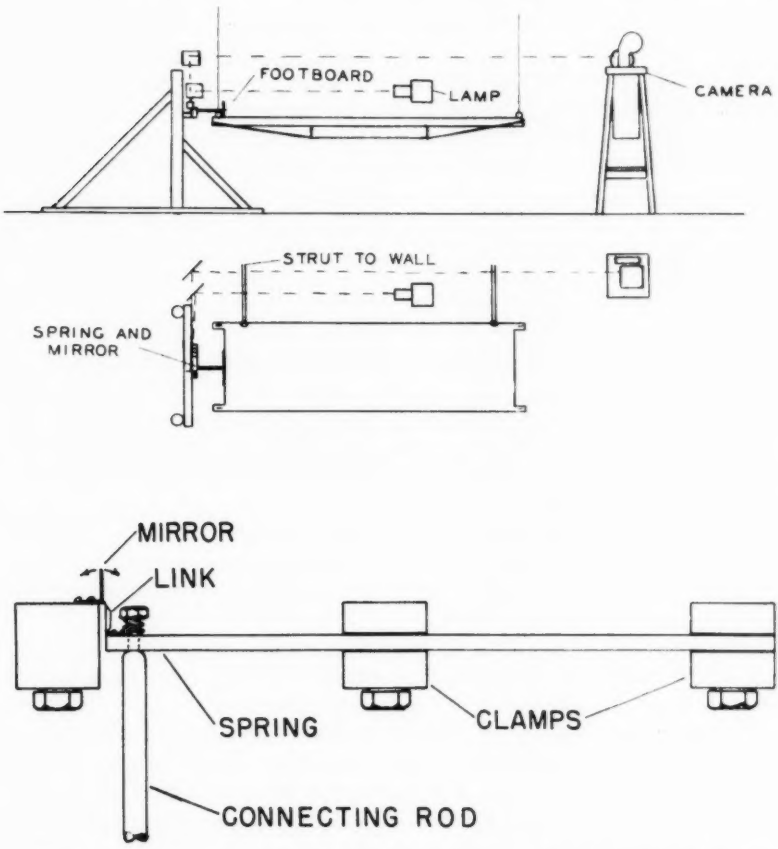

Fig. 1. Apparatus, from the side, and from above. Details of spring and mirror; the left clamp may be moved along the spring to change its characteristics. Pushed about 3 inches from its center of balance the suspended table rests against the spring.

of which is pivoted to the spring support. The other end is connected to the moving end of the spring by a short link.

The optical record is secured in the usual manner, the table's movements being magnified about 8000 times.[1]

[1] The smaller value for magnification given in preliminary reports, Starr et al., 1938a, b, was the result of an error.

The moving film camera carries film 6 or 12 cm. in breadth, the smaller size serving for most purposes. A ruled glass plate, mounted in front of the camera lens and producing parallel lines 1 mm. apart on the film, simplifies the estimation of the altitude of the waves.

Physical Properties of the Apparatus. Calibration. A weight is suspended from a cord attached to the ceiling, making a simple pendulum. When hanging free this cord lies about 1 meter beyond the foot plate and in the projected midline of the table. To apply a known force the suspended weight is swung back and laid against the vertical surface of the foot board (see fig. 1) which, providing no vertical support, prevents the return of the pendulum to its mid position. The cord now makes an angle, θ, with its former vertical position. The component of force, P, directed horizontally against the foot plate for any weight, w, is, when θ is small:

$$P = w \sin \theta$$

Before calibration the table is weighted with 125 lbs. of iron bars. It is then pushed off balance and allowed to come to rest. This is repeated, the initial impulse being applied in alternate directions until 6 to 10 estimations of the rest point of the image in the light spot have been secured. The individual rest points seldom differ by more than 3 mm. and the mean is utilized.

This procedure is repeated after the calibrating weight has been applied, and again after it has been removed; a safeguard necessary because too vigorous a blow on the table sometimes alters the rest point during the calibration.

Over the range in use, different forces cause a proportional displacement of the light spot image, the theoretical curvature is too small to be detected. The amount of weight on the bed makes no difference.

In clinical work the spring of our apparatus is adjusted so that a static force of 280 grams displaces the image 1 cm. Throughout the experiments described in this paper this adjustment has been used except where a statement to the contrary is made. For animal experiments we sometimes readjust the spring to secure more amplitude.

Period of vibration. The behavior of the table after a single blow is shown in figure 2. The frequency varies with the weight on the table as follows: 50 lbs., 15.5 vibrations per sec.; 100 lbs., 12.5 per sec.; 150 lbs., 10.5 per sec.; 200 lbs., 9.5 per sec. when the table is weighted with iron bars.

Damping. There is no mechanical damping in the system. Damping is provided by the human body as was recognized by Abramson (1933).

Mechanical interference. Extraneous vibrations appear in the records. These can be produced by striking the table, its supporting wires, the

floor, or the ceiling. Walking near the spring supports causes some disturbance, as does the movement of the elevators in the building. Most of these interfering vibrations have a rapid period, between 50 and 100 per sec. Some vibrations of this type are usually present. Occasionally they interfere, usually they cause only slight blurring of the edges of the record.

Physical Properties of the Human Body. Period of vibration. Figure 2A shows the effects of a single blow on the table weighted with 115 lbs. of iron bars and a comparable experiment (fig. 2B) with the bars replaced by a fresh cadaver of similar weight. The period of vibration has been completely changed by the presence of the human body. A similar result was obtained on six additional cadavers.

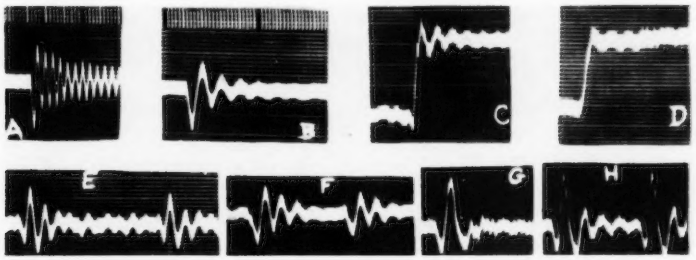

Fig. 2. Vibration properties of the table and of the human body. Records ½ actual size. A. Record when table, weighted with 115 lbs. iron bars, was tapped once. B. When the table, weighted by a fresh cadaver of 115 lbs. was tapped once. C and D. Table occupied by cadavers 4 and 6, calibrating weight lifted off. E. Record of patient H, obtained 2 months before death: F. Same subject ½ hour post mortem, tapped on head twice. G, H. Records obtained by application of mechanical impact maker (fig. 3) to head of cadaver 5. The time record on A and B, largest interval = 1 sec., applies to all the records.

This striking change of vibration properties causes us to assume that we are dealing with a situation analogous to that described by Timoschenko (1929) in which two systems, each of different vibration frequencies, are coupled together by an elastic restraining force. Under such circumstances, if the coupled system is started vibrating, two frequencies appear neither of which corresponds to the frequencies inherent in each part when they vibrated separately.

In our system, with a body on the table, only one vibration has a measurable amplitude. Two considerations indicate that this period must be more dependent on the properties of the body than on those of the apparatus. First the mass of the body is much greater than that of the table. Second, when the table carries no extra weight, or when it is weighted with iron bars, adjustment of the spring to give first the lowest, then the highest tension possible, more than doubles the frequency. With a cadaver on

the table, the same change has practically no effect on the frequency. Obviously, therefore, the physical properties of the body dominate those of the apparatus.

Seven still warm cadavers placed on the table, gave a frequency of 5.0; 5.4; 5.4; 5.9; 5.9; 6.0 and 6.7 cycles per second respectively, the average being 5.76.

If the pulse rate is slow enough the same frequency can be demonstrated on living subjects by striking them a series of single taps on the head. Certain of these impacts will chance to fall between the cardiac complexes. It is hard to secure vibrations entirely unaffected by the cardiac impacts, but the frequency can be estimated approximately in almost every subject. For the past year this has been done routinely. A random sample of 31 of these living subjects showed an average frequency of 5.72 per second, with a standard deviation about the mean of 0.64, values practically identical with the results obtained on cadavers.

Damping. Figure 2 shows the great increase of damping which occurs when a subject is placed on the apparatus. This was recognized by Abramson (1933). But, to obtain a perfect record of the forces generated by the movements in the circulation, our apparatus should be critically damped, i.e., the system should respond to a force applied in negligible time and without overshooting. With a body in the bed we do not have critical damping. The degree of overshooting can be estimated from the cadaver experiments. Deflected by a single tap, figure 1, B and F, or by changing the force applied, figure 1, C and D, the light spot image overshot the resting position by an amount which varied from 30 per cent to 50 per cent of the original deflection, and caused diminishing after-vibrations in the body's period which persisted for from 0.3 to 0.6 sec. in the various experiments. In the records of 20 living patients tapped on the head between heart beats the average overshooting was 40 per cent of the preceding deflection with a standard deviation about the mean of 8.7 per cent. It is obvious therefore that our records, as well as those of our predecessors in this field, cannot be regarded as giving a completely true account of the forces which originate them.

Lag. A single tap on the head of a subject on the table, recorded both by direct interruption of a light beam and by the motion imparted to the table showed that the lag was less than 0.02 sec. As this seemed to be negligible for our purposes we did not conduct experiments to estimate it more accurately.

On the Nature of the Errors and their Correction or Avoidance. The apparatus pictured in figure 3 was designed to test our recording system, in both its mechanical and corporeal aspects, by applying known forces to the head of a cadaver lying on the table, in order to permit a comparison between such forces and the record obtained.

Four cams were designed so that the curves would simulate records obtained from both normal and diseased hearts. A variable speed motor permitted us to change the relationships between the forces generated by the cam and the phases of the body's vibrations.

In a typical experiment this impact-maker was applied to the head of a fresh cadaver. A record was obtained while the cam was rotated slowly

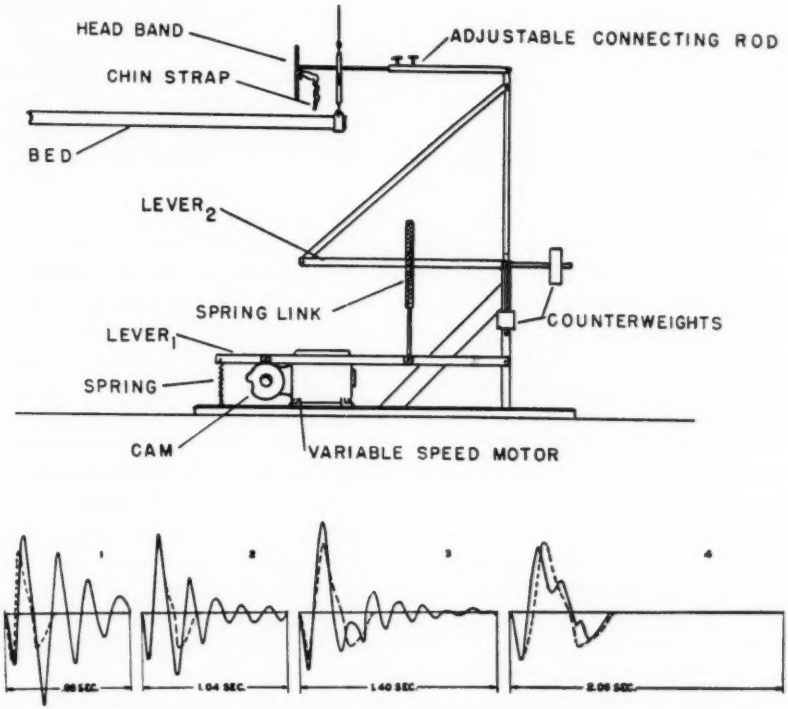

Fig. 3. Diagram of mechanical impact maker, and an analysis of the records obtained. Dotted lines = diagrams of forces applied, solid lines the records secured at different speeds of the impact maker applied to the head of a cadaver within 30 min. post mortem.

and the motor was then run at increasing speed. Typical examples of the resulting record are shown in figure 2. In figure 3 similar records (solid lines) have been plotted for comparison with estimates of the force applied (dotted lines) derived from the shape of the cam and a calibration.

The demonstration of the errors is very striking. The forces causing the initial downstroke, wave I, are quite well reproduced; the largest upstroke, wave J, tends to be somewhat too large. After this the system is thrown

into a damped vibration which persists after the application of force has ceased and which is apparently identical with that seen in cadavers struck a single blow (fig. 2, B, F). Obviously the vibrations inherent in our system interfere with the correct recording of the forces applied. As the trouble seems to be a property of the body we have little expectation of improving it by perfecting our mechanical apparatus.

Mathematical correction of the records was considered. O. Frank (1911) solved a similar problem in his study of physiological recording apparatus. We plotted the correction obtained from Frank's formulae (1911) and were disappointed to find that very little improvement resulted. His theory is apparently not applicable to our conditions. We think our mathematical problem could probably be solved, but the correction would be laborious to apply and we have not attempted a solution at this time.

An alternative is to use only that part of the curve where distortion is at a minimum. Wave I is always a good record of the force applied except where, at very rapid rates, HI interrupts a large after-vibration moving the body in the opposite direction, a situation easily recognized. We would have based our method on wave I had it not been too difficult to place the base line accurately in some records. If deflections on both sides are used this error is minimized.

The altitude of the largest upward deflection, J, is usually greater than that to be expected from the force applied. This is because it may have two components: 1, the force applied by the cam, and 2, the rebound from the previous downward deflection, I. When I and J are in phase with the natural vibration of the body, the height of J can be corrected for overshooting by subtracting 40 per cent of the height of I. Thus corrected its average height approximates the force applied as in figure 3, no. 2 and 3.

The areas under the calculated and recorded curves of waves I and J agree rather better than their heights, the effect of the body's period tending to add in one place what it subtracts in another, as in figure 3.

Records of mechanical impacts at increasing rates have been obtained 12 times on 4 fresh cadavers, the magnitude and form of the impacts being varied. From this experience several statements can be made. The records from which the drawings of figure 3 were made show our apparatus at its worst, for the impacts there recorded were of a size found only in large healthy young men and, at high speed, were delivered at a rate rarely encountered in resting subjects. Impacts of smaller magnitude, as found in older or lighter persons, produced smaller after-vibrations more rapidly damped out. The forces generated by cams which simulated the more rounded impact curves produced by diseased hearts were reproduced more successfully than the pointed normal curves. But the principles demonstrated in figure 3 apply to all cases. Unless after-vibrations interfere,

wave I gives a reasonably accurate record of the force applied. Wave J varies somewhat more but, when corrected, its altitude gives a reasonably true record. But after these large waves have passed their after-vibrations dominate the picture.

An additional demonstration of this fact is to be found in figure 2. Comparison of the record of patient H, two months ante mortem (fig. 2 E), with that obtained by tapping him on the head ½ hour post mortem (fig. 2 F), demonstrates that the descending part of the systolic complexes of the first record is nearly identical with the damped vibrations set in motion by the single impacts delivered after death.

Taking and interpreting records. The subject lies supine on the table, his feet in firm contact with the foot board. We allow him one or two pillows. He must remain relaxed and motionless. After 10 or 15 minutes' rest the operator chooses a moment of quiet and photographs the bed's motion.

If the subject is not in contact with the foot board the form of the record is slightly altered, the peaks becoming lower and more rounded and some of the minor features like the diastolic wave tending to disappear.

When the subject was firmly in contact with the foot board, further fixation of his body to the bed by straps did not alter the record in two experiments.

Reading the records. As movements made by the subject affect the record artefacts are common. Only the features that are regularly repeated deserve any attention.

The *amplitude* of the cardiac complexes varies with the phases of respiration, the lowest occurring during expiration. Theoretically amplitude should be estimated throughout a respiratory cycle, but in most instances this is unnecessary. In 50 records we averaged the amplitude throughout the respiratory cycle and compared it with the average amplitude of the highest and lowest complexes. The latter estimation proved to be only 4.2 per cent different from the former, an error within that of reading the records. Therefore we estimate amplitude by inspecting the whole record, selecting representative high and low complexes, measuring and averaging the height of their waves. It is not always easy to determine the base line with confidence so we place more dependence on the distance between the valley I and the peak J, than the deflections of either from the base line.

The *form* of the record may be described in terms of the waves, giving them the letters shown in figure 7, or in terms of lines between the peaks and valleys HI, IJ, JK, etc.

In figures 4 B and C two *normal records* are shown with simultaneous records of the heart sounds and the electrocardiogram. The form of these ballistic records is essentially similar during systole but somewhat different

during diastole. The diastolic differences are artefacts; in Sta. the impacts start his body vibrating; in Sch. they are out of phase and the after-vibrations do not appear.

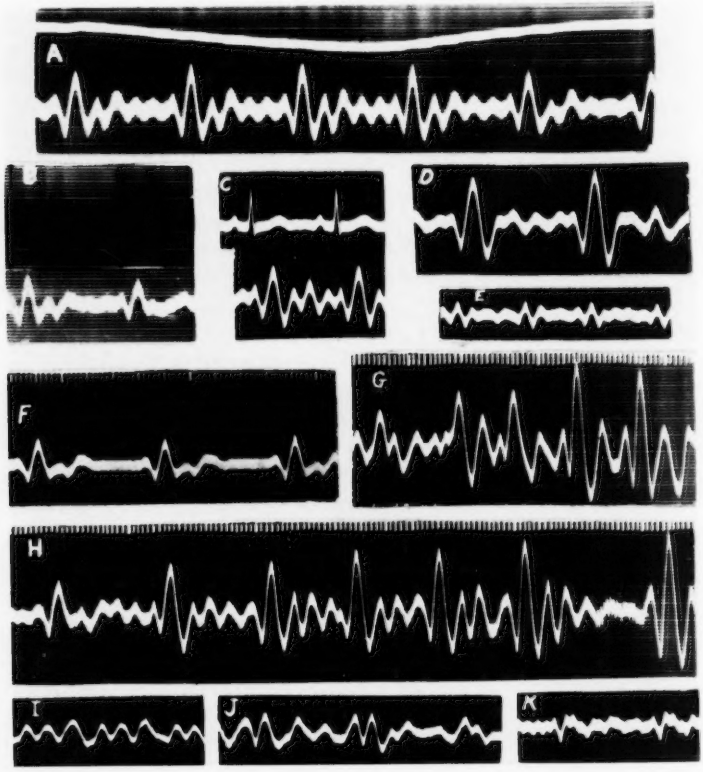

Fig. 4. Ballistocardiograms reduced to ½ actual size. A. Record of normal subject Sta., wt. 185 lbs., with simultaneous record of respiration, moving downward during inspiration. B, of normal subject Sch., wt. 155 lbs., with simultaneous record of heart sounds. C, of Sta. with simultaneous electrocardiogram. D, of normal subject Fiel., a professional wrestler, wt. 250 lbs. E, of a normal baby, wt. 25 lbs. F, from Sch. basal, just before exercise. G, from the same 2 min. after running up eight flights of stairs. H, from the same 4 min. later. I, from patient C. B.P. 220/140, an abnormal record. J, from patient B. B.P. 164/100, an abnormal record. K, from patient P, an abnormal record. The time record on H applies to all records, largest interval = 1 sec.

Theoretical considerations. To relate the properties of the ballistic curves to the cardiac output two courses were open to us. We could

proceed empirically, comparing our curves with the results of estimations of cardiac output by another method and developing a formula on the basis of the relationship. Also we could proceed theoretically, by attempting to calculate the size and shape of the impact curve, if the cardiac output were known, and other necessary factors estimated or assumed. We finally used both methods. The theoretical approach clarified our ideas concerning the relation of the ballistic curves to the blood's velocity, the cross section of the great arteries, and the events of the cardiac cycle, and we were both surprised and gratified to find that, after making the assumptions which appeared to us most reasonable, a cardiac output formula was secured which gave results agreeing well with those secured by the ethyl iodide method. However it was always apparent that some of our underlying assumptions were of doubtful accuracy and that others, equally defensible, would yield a somewhat different result. Finally, therefore, we developed our cardiac output formulae in terms suggested by the theoretical approach. But the stronger evidence for their accuracy lies, not in the reasoning by which they were derived, but in the agreement of the results with those obtained by ethyl iodide.

As our theoretical conceptions determined the form of our final equation they will be considered first.

The *physical factors* which control the deflections of the table are three: the elasticity of the apparatus and the body, the mass of moving blood involved, and the rate of change of its velocity, i.e., its acceleration. Neglecting the elasticity, we have

$$F = m\frac{dv}{dt} \tag{1}$$

where F is the force, m the mass of blood and $\frac{dv}{dt}$ its acceleration.

On this basis we have constructed an approximate curve of the chief forces exerted by the moving blood of a normal man during a cardiac cycle. It is simpler to determine the shape of the curve first and to assign numerical values later.

The shape of the theoretical impact curve. Curves of the velocity of the blood in the ascending aorta of dogs have been published by Broemser (1928) and by Machella (1936). In figure 5A1 we have reproduced such a curve redrawn in arbitrary linear coördinates from Machella's (1936) data. We have assumed that the shape of this curve holds for the normal human subject and that it applies to the pulmonary artery as well as to the aorta. The mean velocity during ejection, V_a, according to this curve is 1.1 divisions of ordinate.

From figure 5A1 we have estimated the acceleration $\frac{dv}{dt}$ by measuring

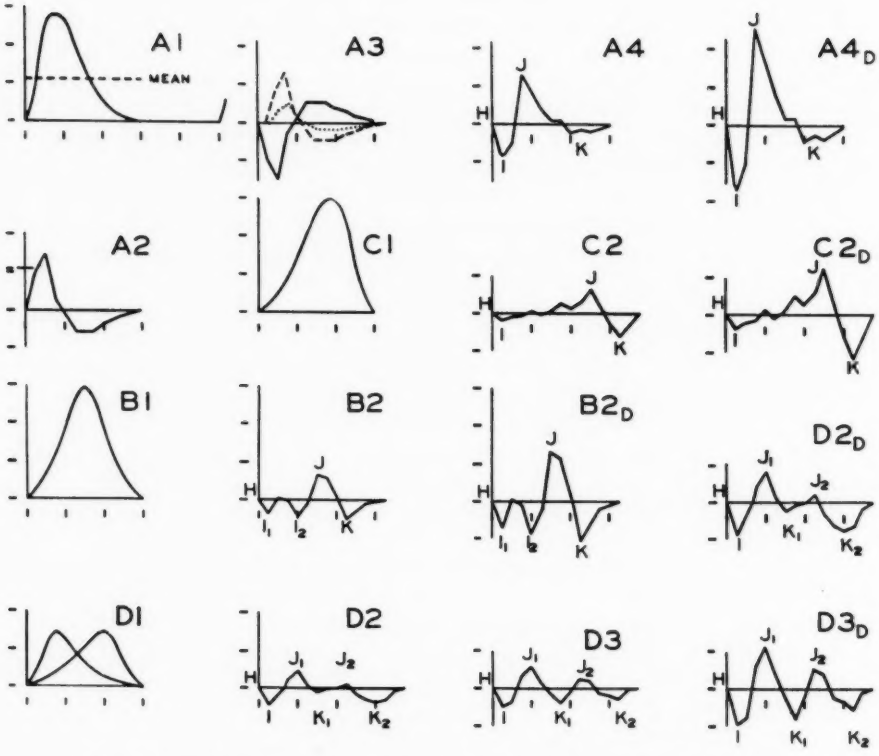

Fig. 5. Calculation of ballistic records from forces generated by movement of blood in aorta and pulmonary artery during systole.

A 1, normal curve of blood velocity in aorta. Machella's data (1936) redrawn to linear coördinates. A 2, rate of change of A 1, i.e., acceleration or dv/dt. A 3, the three chief forces, see text. A 4, resultant of forces plotted in A 3. A 4 D, same as A 4 but with vertical scale doubled to give the proportions seen in normal ballistocardiograms.

B 1 and C 1 are assumed abnormal curves of blood velocity in both aorta and pulmonary artery. From them B 2 D and C 2 D were derived as was A 4 D, the form of each is characteristic of a type of ballistocardiogram found in certain patients, the double I, and the late downstroke types respectively.

D 1 shows assumed velocity curves in the aorta and pulmonary artery when one heart is weak, the other normal. D 2 D, is the calculated ballistic curve if the left heart is weak, D 3 D, the same when the right heart is weak. These M shaped types are frequently seen in patients.

The small letters at the peaks and valleys are those which we have used to identify various parts of the ballistic records. See also figure 7.

the difference in velocity at short intervals. In figure 5A2 we have plotted this acceleration, again in arbitrary coördinates, 1 division representing a change of velocity of 1 division of ordinate per $\frac{1}{4}$ division of abscissa. This value of $\frac{1}{4}$ division of abscissa is 1/20 the duration of the cardiac cycle.

The cardiac recoil. All the blood discharged from both sides of the heart during systole is involved in this impact and we have assigned a value of unity to this mass of blood. Obviously the total mass involved is greater than this for part of the heart's muscle moves headward also. We see no way of estimating this latter mass and have neglected it. Since the force of this impact is exerted feetward, initially, we have estimated it by multiplying the curve of acceleration (fig. 5A2) by a mass factor of -1 and plotted the resulting curve on an arbitrary linear scale as the solid line of figure 5A3. This curve indicates our conception of the forces which would be exerted on the body if, after the heart's contraction, all the blood squirted from the head end without encountering any resistance.

The aortic impact. A brief interval after issuing from the heart the blood reaches the aortic arches and the headward movement of most of it is arrested causing a second impact. The mass involved consists of all the blood discharged from the right heart and most of that from the left but a certain portion continues headward up the carotids to be arrested later. We arbitrarily consider this portion to be $\frac{1}{3}$ of the systemic output per beat. The mass involved in the aortic impact is therefore $\frac{5}{8}$ of that in the heart's recoil.

Because of the short distance traveled the velocity curve has been assumed to be similar to that of the blood leaving the heart although the curvature of the arch will warp the longitudinal velocity somewhat, producing an error hard to evaluate.

To represent this impact, we have multiplied the ordinates of figure 5A2 by $+\frac{5}{8}$ and plotted them as the broken line in figure 5A3, starting $\frac{1}{25}$ of the duration of the cycle after the beginning of ejection.

The aortic recoil. After striking the aortic arch $\frac{2}{3}$ of the systemic blood is accelerated feetwards and the recoil from this motion is a force exerted headwards. We have, therefore, multiplied the ordinates of figure 5A2 by $+\frac{2}{8}$ and plotted them as the dotted line in figure 5A3, starting $\frac{3}{50}$ of the duration of the cycle after the start of the cardiac recoil curve.

Other impacts. As the blood slowly loses its longitudinal velocity in the lungs and in the peripheral circulation a series of impacts must take place. These must be much smaller than the three impacts mentioned above. First, the masses of blood involved at any one time are much smaller; second, acceleration is much smaller as the periphery is approached, and finally, these impacts are delivered in opposing directions. Therefore we believe that their effect on our ballistic curves is negligible.

Small impacts occurring in diastole and just before ejection are a feature of our records and their significance will be considered later.

The shape of the ballistic curve. Evidently, after a single systolic discharge, the body is subjected to a series of overlapping forces of which three, the heart's recoil, the aortic impact, and the aortic recoil, far overbalance the others in importance. The resultant of these forces will cause the table to be deflected. This resultant is the algebraic sum of the curves of figure 5A3 for each interval of time. This has been plotted as figure 5A4, and redrawn to an appropriate scale in figure $5A4_D$. The shape of this curve closely resembles the normal ballistocardiogram.

The size of the impact curve. While the ordinates in figure 5A4 bear a definite numerical relation to the mean velocity in figure 5A1, it must be remembered that for a different shape of ejection curve the relation would be different. The numerical relations for the curve given can be derived as follows.

The mean velocity in the aorta is

$$V_m = \frac{S_v}{A \times E} \tag{2}$$

where S_v is the stroke volume in cubic centimeters, i.e., the blood issuing from one side of the heart at each systole, A the cross section of the aorta in square centimeters, and E the duration of ejection in seconds. Since in figure 5A1, $V = 1.1$ divisions, we have

$$1 \text{ div.} = \frac{V_m}{1.1} = \frac{S_v}{1.1 \times A \times E} \tag{3}$$

the units of velocity being centimeters per second.

It follows from the construction of figure 5A2 that in this curve

$$1 \text{ div.} = \frac{S_v}{1.1 \times A \times E \times \frac{C}{20}} \tag{4}$$

C being the duration of the cycle in seconds, and the units of acceleration being square centimeters per second.

In figures 5A3 and 5A4 the ordinates represent the product of the mass involved and the acceleration, i.e., the force. The specific gravity of blood being approximately 1, the mass in grams is approximately equal to the volume in cubic centimeters, and we have

$$1 \text{ div.} = 2S_v \frac{S_v}{1.1 \times A \times E \times \frac{C}{20}}$$

or

$$1 \text{ div.} = \frac{36.4(S_v)^2}{A \times E \times C} \tag{5}$$

the units being gram centimeters per second per second, or dynes. Dividing the right side by 980 we have

$$1 \text{ div.} = \frac{(S_v)^2}{27\,AEC} \tag{5A}$$

the units being grams. Therefore if the ordinate of a wave in figure 5A4 is y divisions, the force will be

$$F = \frac{y(S_v)^2}{27\,AEC} \tag{5B}$$

According to an empirical formula given by Bazett (1920)

$$\text{Systole} = 0.4\ \sqrt{C} \tag{6}$$

Allowing $\frac{1}{6}$ of the duration of systole for the pre-ejection phase of cardiac contraction we have for the duration of ejection

$$E = 0.34\ \sqrt{C} \tag{7}$$

Substituting this value for E in equation 5B, we have

$$F = \frac{y(S_v)^2}{9.2\,AC^{\frac{1}{2}}} \tag{8}$$

By applying the calibration of the table, 1 mm. = 28 grams, we find that the deflection on the record in millimeters due to a wave of y divisions altitude in figure 5A4 is

$$F_r = \frac{y(S_v)^2}{257\,AC^{\frac{1}{2}}} \tag{8A}$$

To assign numerical values to the constants in equation 8A we must have certain data from a normal subject. Thus on a certain day Sta. was 43 years old, his height 6 ft., his weight 185 lbs., his heart rate 73/min., and his stroke volume estimated by the ethyl iodide method (Starr and Gamble, 1928) was 62 cc. per beat.

From Bazett's data (1935) on the variation of aortic cross section area with age and size of subject we estimate Sta.'s aortic cross section area as 3.64 cm.^{2} We assume that the pulmonary artery was the same size. Substituting the above values in equation 8A we have

$$F_r = \frac{y(62)^2}{257 \times 3.64 \times \left(\frac{60}{73}\right)^{\frac{1}{2}}} = 5.54\,y \tag{8B}$$

For the waves I, J and K, y in figure 5A4 is 0.86, 1.23, 0.12 respectively. Therefore in the theoretical ballistocardiogram for Sta., I = 4.7 mm., J = 6.7 mm., K = 0.7 mm.

Theoretically the areas under the curve in figure 5A4 should be a better measure of cardiac output than the altitudes of the waves. In figure 5A1 the duration of the cycle is 5 divisions. From the value of E in equation 7 we compute that

$$1 \text{ div. of abscissa} = \frac{C}{5} \tag{9}$$

the units being seconds. From equations 5A and 7 we have

$$1 \text{ div. ordinate} = \frac{(S_v)^2}{9.2\,AC^{\frac{1}{2}}} \tag{10}$$

the units being grams. From equations 9 and 10 we have

$$1 \text{ sq. div.} = \frac{(S_v)^2}{46\,A\sqrt{C}} \tag{11}$$

Therefore if the area under a wave is z sq. div., the impact will be

$$FT = \frac{z(S_v)^2}{46\,A\sqrt{C}} \tag{12}$$

the units being gram-seconds. By applying the calibration of the table, we find that the area under a wave on the record, due to a wave with an area of z sq. div. in figure 5A4, is

$$F_r T = \frac{z(S_v)^2}{1290\,A\sqrt{C}} \tag{12A}$$

the units being millimeter-seconds. Substituting numerical values we have

$$F_r T = \frac{z(62)^2}{1290 \times 3.64 \times 0.9} = 0.91\,z \tag{12B}$$

Since the waves in figure 5A4 are roughly triangular we can easily estimate the areas under them.

For I, z = 0.29, for J, z = 0.57. Therefore in the theoretical ballistocardiogram for Sta. the area under wave I is 0.26 and that under wave J is 0.52 mm.-sec.

Ballistocardiograms were made on Sta. before and after the estimation of cardiac output which furnished the data used in this computation. The heights of the waves of representative small and large complexes were measured and averaged. Areas under the waves were estimated by

constructing triangles of approximately equivalent size. The results are recorded in table 1.

This experiment was repeated on six other trained subjects and the theoretical ballistocardiograms were calculated for each in the manner described above. The individual and average results are also in table 1.

Correction of the ballistocardiogram. On page 8 it was demonstrated that, when a cadaver on the table was thrown off its resting position by a single impact the rebound overshot the midline by an amount which averaged 40 per cent of the height of the preceding deflection. Therefore when, as in the normal ballistocardiogram, a downward wave is followed by an upward one, at an interval which coincides with the body's vibrations, the latter must be reinforced by the rebound from the former.

TABLE 1

Comparison between the average altitudes of the ballistic waves, and the values calculated from theory

SUBJECT	WAVE H ALTITUDE FOUND	WAVE I			WAVE J			WAVE K			WAVE I + WAVE J	
		Alt. found	Alt. found corrected	Alt. calculated	Alt. found	Alt. found corrected	Alt. calculated	Alt. found	Alt. found corrected	Alt. calculated	Alt. found corrected	Alt. calculated
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Sta	3.0	5.0	3.8	4.7	8.0	6.0	6.7	4.0	0.8	0.7	9.8	11.4
Sch	2.5	3.7	2.7	6.3	9.3	7.8	9.1	4.5	0.8	0.9	10.5	15.4
Mont	0.5	5.8	5.6	5.9	9.5	7.2	8.3	6.0	2.2	0.8	13.8	14.1
Land	3.0	4.5	3.3	4.2	9.2	8.2	6.0	6.0	2.3	0.6	11.5	10.2
Burt	2.5	6.0	5.0	5.4	13.0	10.6	7.7	6.2	1.0	0.8	15.6	13.1
Fiel	2.0	6.5	5.7	6.1	12.0	9.4	8.7	6.0	1.2	0.8	15.1	14.8
Luk	2.0	7.5	6.7	6.0	12.5	9.5	8.5	6.7	1.7	0.8	16.2	14.5
Averages	2.5	5.6	4.7	5.5	10.5	8.4	7.9	5.6	1.4	0.8	13.2	13.4

When the two waves are in phase, correction may be made by subtracting 40 per cent of the height of the previous deflection, the remainder being a better representation of the actual force applied. This correction has been applied to the wave heights found and the results are given in table 1. A similar correction was considered for the areas, but from the results recorded in figure 3 we concluded that the interfering vibration subtracted in one place about as much area as it added in another. Therefore we have made no correction for areas I and J in table 2.

Comparison between values found and theoretical expectations. The results recorded in table 1 show that while in certain individuals the agreement between values found and calculated for waves I and J is only fair, the agreement of the averages is most gratifying. On the other hand

wave K, largely an artefact due to overshooting from the preceding wave, cannot be estimated as accurately.

The data also justify certain conclusions. In some cases differences from theory may be attributed to faulty location of the base line on the records. If another base line is chosen, the agreement is greatly improved. Therefore it is wise to make computations from the sum of two opposite deflections, so that an error in locating the base line causes little difference in the results.

CALCULATION OF THE CARDIAC OUTPUT FROM THE BALLISTOCARDIOGRAM. Having calculated the characteristics of the ballistic waves when the cardiac output was known and the shape of the ejection curve assumed, we can reverse the process, and with the same assumptions, estimate the cardiac output from the ballistocardiogram.

TABLE 2

Comparison between the average of the areas found under the ballistic waves, and the values calculated from theory

SUBJECT	AREA H FOUND	AREA I		AREA J		AREA I + AREA J	
		Found	Calculated	Found	Calculated	Found	Calculated
	mm. sec.	*mm. sec.*	*mm. sec.*	*mm. sec.*	*mm. sec.*	*mm. sec.*	*mm. sec.*
Sta	0.8	0.22	0.26	0.58	0.52	0.80	0.78
Sch	0.6	0.17	0.40	0.65	0.78	0.82	1.18
Mont	0.05	0.20	0.33	0.69	0.64	0.89	0.97
Land	0.13	0.19	0.26	0.56	0.51	0.75	0.76
Burt	0.05	0.24	0.29	0.70	0.58	0.94	0.87
Fiel	0.08	0.33	0.36	0.93	0.71	1.26	1.07
Luk	0.09	0.34	0.45	0.79	0.88	1.13	1.33
Average	0.09	0.24	0.34	0.70	0.66	0.94	0.99

Solving equation (8A) for S_v we have

$$S_v = 16 \sqrt{\frac{F_r A C^3}{y}} \tag{13}$$

where, as before, S_v is the stroke volume in cubic centimeters, y is the altitude of a given wave in figure 5A4 in scale divisions, F_r the altitude of the corresponding wave on the ballistocardiogram in millimeters, A the aortic cross section in square centimeters, and C the length of 1 heart cycle in seconds.

Therefore to estimate cardiac output from the altitude of wave I, in millimeters

$$F_r = I \text{ and } y = 0.86.$$

Then

$$S_v = 17.3 \sqrt{I A C^3} \tag{13A}$$

and similarly for wave J, F_r now $= J$ and $y = 1.23$.

Then

$$S_v = 14.4 \sqrt{J A C} \tag{13B}$$

and combining 13A and 13B to obtain a formula in which an error in locating the base line will make little difference, and rounding out

$$S_v = 7 \sqrt{(3I + 2J) A C} \tag{14}$$

To estimate cardiac output from the wave areas we can solve equation 12A for S_v, obtaining

$$S_v = 36 \sqrt{\frac{F_r T A \sqrt{C}}{z}} \tag{15}$$

where S_v is the stroke volume in cubic centimeters, z is the area under a given wave in figure 5A4 in square divisions, $F_r T$ is the area under the corresponding wave on the ballistocardiogram in millimeters-seconds, and A is the aortic cross section in square centimeters.

Then, recalling that, from the calibration of the table, 1 mm.-sec. = 28 gm.-sec.,

If $F_r T = \int I dt$ in mm. sec., then $z = 0.29$ and

$$S_v = 67 \sqrt{\int I dt \, A \sqrt{C}} \tag{15A}$$

and if $F_r T = \int J dt$ in mm. sec. then $z = 0.59$ and

$$S_v = 47 \sqrt{\int J dt \, A \sqrt{C}} \tag{15B}$$

and combining 15A and B and rounding out

$$S_v = 33 \sqrt{(2 \int I dt + \int J dt) \, A \sqrt{C}} \tag{16}$$

From the mathematical viewpoint the integral formula (no. 16) is superior to that derived from the waves altitudes (no. 14), but from practical considerations the reverse is true. Height is more easily measured than the area under the waves. Therefore in this early stage of the subject we have given more attention to the simpler method.

Therefore, to calculate cardiac output from any normal ballistocardiogram we are usually satisfied to measure the altitudes of waves H, I and J, apply a correction for overshooting if the waves are in phase, ascertain the aortic cross section area for the subject's age and surface area from Bazett's formula (1935), estimate the cycle from the heart rate, and apply formula 14.

When the impact waves have an abnormal form, the cardiac output formulae (no. 14 and 16) may not be applied. However one may develop other formulae by starting with the assumption that the velocity curve is of an abnormal shape and repeating the mathematical process outlined on pages 14 to 19. Figure 5 shows the resultant ballistic curves obtained

from various types of abnormal velocity curves. Ballistic records corresponding to all these types have been obtained in patients with heart disease, types D2 and D3 being encountered quite frequently, types B2 and C2 being rare. Using procedures and assumptions identical with those outlined, a cardiac output formula may be developed for each type which differs from formula 14 only in the numerical constants.

Thus for curves of the C2 type we obtain approximately

$$S_v = 15 \sqrt{(J + K)A\,C} \tag{18}$$

For the B2 type

$$S_v = 15 \sqrt{(I_2 + J)A\,C} \tag{19}$$

For *D1* and *D2*, using alt. I and J_1, the constants are 16 and 15 respectively. For the normal records this constant is 11.

Therefore to estimate cardiac output when the ballistocardiogram is abnormal one must first decide which of the abnormal types he is dealing with and then apply the corresponding formula. Fortunately the constants of the different types are very similar. Even if one mistook the type, the amplitude of the most prominent deflections on the record, used with any of the constants, would give an approximately correct result. More difficulty comes in evaluating forms intermediate between the extremes cited in figure 5 and the normal. Until more data can be accumulated the best chance of success would be to choose a constant half way between the normal and abnormal, i.e., 13.

EXPERIMENTS. *Reproducibility.* The series described below afforded a comparison between duplicate ballistocardiograms obtained about 1 hour apart on 30 individuals. The altitudes of the 4 largest waves of representative high and low complexes were measured and the sum taken as an index of amplitude. The average deviation of each duplicate from the mean of the pair was 3.8 per cent.

In subjects Sta. and Sch. records were made in the post absorptive state on four days at weekly intervals. In Sta. the average deviation of the amplitudes of each day from the mean of all four days was 8.7 per cent, for Sch. 6.0 per cent.

As these data indicate the records are highly reproducible and characteristic. Especially if the form is abnormal, the subject can usually be recognized by a glance at his record.

Comparisons between cardiac output, estimated from the ballistocardiogram, and by the ethyl iodide method. The 30 subjects, 7 of them physicians, the rest hospital ward patients, ranged from 14 to 63 years of age and from 72 to 250 lbs. in weight. None of the patients had any pulmonary disease or evidence of aortic or mitral regurgitation. Ballistocardiograms were obtained before and after a duplicate determination of cardiac output by

the improved ethyl iodide method (Starr and Gamble, 1928). The assistance of a trained chemist being available the ethyl iodide samples were analyzed by Cool's method (1932).

To avoid unconscious bias the ballistocardiograph film was never developed until the result obtained by the ethyl iodide method was written in the record book. Then the ballistocardiograms were inspected, representative high and low complexes were selected and measured. The correction for overshooting, subtracting 40 per cent of the height of the

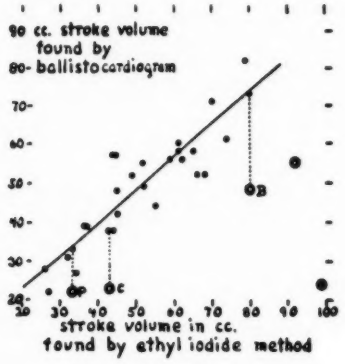

Fig. 6

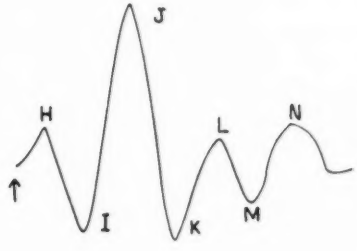

Fig. 7

Fig. 6. Comparison between cardiac st.. ke volume in cubic centimeters calculated from duplicate ballistocardiograms and from duplicate estimations by the improved ethyl iodide method. The small solid dots indicate that the ballistic record had the normal form. The line is the best line, regression y on x, for the 25 small solid dots. The large dots identified by letters represent values obtained on patients with the abnormal ballistocardiograms shown in figure 4 by using formula 14; the connected small hollow dots give the corresponding values obtained when the proper formula was applied (see text). The large dots without adjacent letters represent the two results omitted from the statistical analysis (see text).

Fig. 7. Diagram of commonest form of the normal ballistocardiogram with the letters used to identify the component parts. The arrow indicates the beginning of electrical ventricular systole.

preceding wave, was applied rigidly to avoid bias, although the shape of some of the complexes would have justified leaving it out with slight improvement to the results. Formula 14 was used to estimate cardiac output.

The results are shown in figure 6. The values obtained on five cases which deserve special discussion are shown as big dots and will be considered later. The line is the regression (y on x) of the best 25 cases. The correlation coefficient of these is 0.86 with a standard deviation of 0.052. The results obtained from the ballistocardiogram average 2 cc. per beat,

3.9 per cent, smaller than those obtained by ethyl iodide, and the standard deviation of the difference in the same individual is 14.3 per cent. The standard deviation of the difference between two pairs of duplicates, done on the same individual, on the same day, by the ethyl iodide method is 11.2 per cent (Starr, Gamble, Margolies, Donal, Joseph and Eagle, 1937), a value not significantly different from 14.3 per cent. Therefore the scattering of the results would have been very similar if all had been obtained by the ethyl iodide method.

Of the five cases whose values have been shown as large dots in figure 6, three gave the abnormal ballistocardiograms illustrated in figure 4 G, H, and I, so the erroneous results obtained from formula 14 were to be expected. The M shaped form of patients B and C justifies the use of formula (19) and the omission of the correction for overshooting. When this method is used the agreement is greatly improved, 38 and 73 cc. per beat being estimated from the ballistocardiogram and 43 and 80 cc. found by the ethyl iodide method. The curve of patient P has a flattened or notched J, a form intermediate between the M shaped and the normal. In this case the use of formula 19 overcorrects the result, 40 cc. per beat being calculated, 33 cc. found by ethyl iodide. The intermediate constant, 13, gives a result of 33 cc. per beat.

The discrepancy in the two remaining cases is characterized by the fact that the values found by ethyl iodide, 99 and 92 cc. per beat, are abnormally high, those found by the ballistocardiogram being much lower. Any comparison between results obtained by the two methods is based on the assumption that the subjects can breathe through respiratory apparatus for ½ hour with the same equanimity that they attain lying relaxed on the table. One of the individuals cited was a neurotic, the other a very nervous case of malignant hypertension and we suspect that excitement increased their cardiac output while they were breathing through the respiratory apparatus.

In table 3 is shown the agreement obtained when cardiac output was calculated by the integral formula (no. 16) in our 7 trained subjects.

Animal experiments. To obtain information concerning the effect of abnormal cardiac action on the ballistocardiogram experiments were conducted on 8 dogs anesthetized with chloralose. The chest was opened under artificial respiration, the pericardium divided, its edges being sewn to the edges of the spread incision to permit easy inspection of the heart. Carotid and femoral venous cannulae were inserted and the dog and dog board were placed on the ballistocardiographic table.

Figure 7 gives a typical result following asphyxia obtained by stopping the respiration pump. The record before asphyxia was not quite normal, judged by human standards, doubtless due to the operation. Soon after the pump was stopped, the ballistics changed to the double I type (see fig.

5B2). Then, as the heart was seen to beat more strongly and blood pressure increased, the curves became of normal form and of greatly increased amplitude. But soon signs of cardiac distress were manifest, the tracing showing a notch in JK and becoming more M shaped. After this, failure rapidly supervened. As the blood pressure collapsed and venous pressure rose, ballistic amplitude diminished, I became smaller and K more prominent and later the tracing becoming of the late downstroke type (fig. 5C2).

In another experiment the ballistic record was compared to the record obtained from a Cushny (1897) myocardiograph photographed on the same film. There is obvious correspondence between the amplitudes under conditions of cardiac stimulation and depression.

In four other experiments the aim was to damage the right ventricle without injuring the left. A soldering iron was heated red hot and laid

TABLE 3

Comparison between cardiac stroke volume calculated from the ballistocardiogram and estimated by the ethyl iodide method in subjects familiar with laboratory procedures

SUBJECT	CALCULATED FROM CORRECTED WAVE ALTITUDES (FORMULA 14)	CALCULATED FROM WAVE AREAS (FORMULA 15)	ESTIMATED BY ETHYL IODIDE
	cc.	*cc.*	*cc.*
Sta.	56	60	62
Sch.	52	51	66
Mont.	58	59	65
Land.	60	57	61
Burt.	56	59	59
Fiel.	71	73	70
Luk.	82	71	79

repeatedly on the surface of the right ventricle until the whole was charred. Section of the wall taken after the experiment showed a destruction of muscle which extended entirely through the thickness of the wall in some places, half way in most places and was more superficial in small areas. After such damage the record showed the M shaped tracings illustrated in figure 8L. The resemblance of these to the derived curves shown in figure 5D2, is obvious. Therefore this abnormality may be attributed to inequalities in the strength of the two sides of the heart.

Discussion. From theoretical reasoning only, Abramson (1933) deduced a formula for estimating cardiac output from the ballistic waves, as follows:

$$2\ Sv\ =\ 99\ \sqrt{y}$$

Sv being the stroke volume and y the double integral $\left(\int_0^T dt \int_0^T F dt = y\right)$

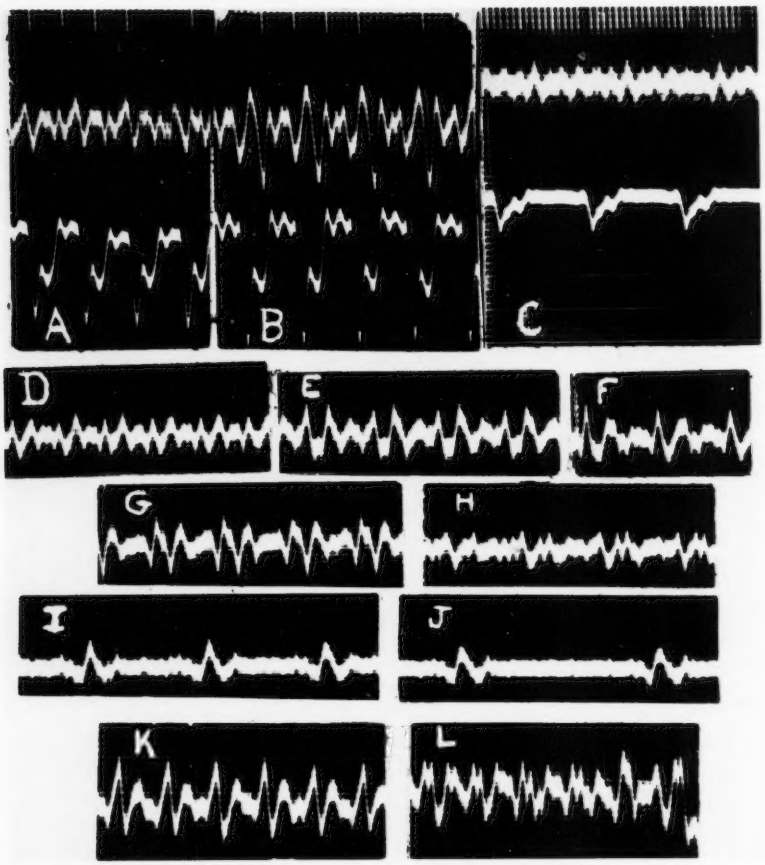

Fig. 8. Animal experiments. Row 1. Dog 8.5 kilo, chloralose, heart exposed, art. resp. Comparison between the ballistic record and the movement of 2 levers tied on surface of right ventricle (Cushny's myocardiograph). A, control, mean B.P. 50 mm. Hg. B, stimulation by intravenous 0.9 per cent NaCl, B.P. 72. C, depression after adding chloroform to inspired air, B.P. 20. Due to stretching of the thread of the myocardiograph, the alignment with the ballistocardiogram is imperfect.

Rows 2, 3 and 4, effect of asphyxia. Dog 11.7 kilo, chloralose, heart exposed. D, control, B.P. 80 mm. Hg, venous pressure 9 cm. H_2O. Other records at intervals of 30 sec. after stopping artificial respiration. E, B.P. 80, V.P. 9, record of double I type (cf. fig. 5B2); F, B.P. 200, V.P. 13, heart is stimulated, record of normal type; G, B.P. 200, V.P. 15, one sided heart failure begins, note notch on JK and M shape; H, B.P. 100, V.P. 18, both sides weaker, note diminished amplitude and M shaped appearance; I, B.P. 60, V.P. 20, both sides now equally weak, note shallow rounded I wave and late K; J, B.P. 40, V.P. 23, Same form, rate slower.

Row 5. Dog 13.8 kilo. Chloralose, heart exposed. *K* before, and *L* after cauterization of right ventricular wall.

The time record at top applies to all records, its longest interval is 1 sec. The records are actual size. The ballistocardiogram spring had been weakened so that the amplitude after a given impact is twice that used for man.

of the positive deflections of the ballistic curves for one cycle, an area corresponding to the area below, minus that above, the base line of our curves. We have performed the double integration graphically on several curves obtained from subject Sta. Difficulty was encountered immediately. The place where one started the integration made the greatest difference in the result as did the location of the base line. So two of the authors working independently got very different results. The best agreement was secured in one of the larger complexes where we estimated a stroke volume of 50 cc. by Abramson's method in comparison with 70 cc. by our own. But Abramson assumed a value of 5 cm.^{2} for both the aortic and pulmonary artery cross section areas and if this is corrected to the figure we used, the discrepancy becomes larger. From a small complex, using Abramson's (1933) formula, one author estimated a stroke volume of 12 cc., another obtained an impossible result, y having a negative sign. By our method this complex indicated a stroke volume of 54 cc.

Examination of Abramson's (1933) basic equations (no. 1 and 2) led us to question the correctness of his method of applying them to our common problem. Thus his equation (1), substituting F for his K to make the symbols conform with ours, may be written:

$$\int_0^T F dt = (m + m_1)\, v = 0$$

He defines: v = the speed of the blood mass; m = the mass of the double stroke volume; m_1 = the other mass set in motion which is later neglected. In this equation $v = O$ over a cycle which seems impossible. The sum of the changes of momentum in the system could properly be equated to zero, but $(m + m_1)\, v$ expresses only a part of the momentum and we doubt if the balance can be neglected.

Having built a chair and recording mechanism with a sufficiently high vibration frequency, Abramson naturally regarded his curves as true records of the forces generated within the body. We regret that our results drive us to another conclusion. There is a definite vibration frequency when a body rests on our apparatus. After-vibrations at this same frequency are plainly visible in Abramson's records also, so we believe that his apparatus does not avoid the difficulty. Therefore when waves of small amplitude follow waves of larger amplitude, their areas are highly unreliable indices of the forces applied in his, as in our own, records. With these difficulties in mind a suitable formula might be constructed to relate the cardiac output to the double integral of the early ballistic waves, but it would be so difficult to apply that we have not attempted it.

One must pay tribute to the great nicety of construction of Abramson's apparatus but the question must be raised whether the same result cannot

be secured by simpler means. Abramson's records are much like those obtained by our far simpler device.

We make no claim that our method will estimate cardiac output with high accuracy. Its results agree with those obtained by the ethyl iodide method almost as well as the results of the latter procedure agree with each other. The ethyl iodide method, applied to normal resting subjects, gives the same average result as is obtained by other modern methods (Donal, 1937). This agreement, obtained by methods based on very different physiological principles, is the best evidence available for their approximate accuracy. But since they cannot be tested by estimating known quantities, their accuracy cannot be stated in mathematical terms, and all of them should be regarded as very crude.

Without attempting to catalogue the possibilities of error or to define all the limitations of the method, especially in pathological conditions, some of the uncertainties of our procedure must be discussed.

Two persons independently selecting and measuring typical complexes may obtain results which differ by 15 per cent in certain instances.

When a body lies on our table perfect transference of the impacts from his heart and aorta to our record is not to be expected, the trouble lying chiefly in the body's tissues. The correction for the imperfections in the system cannot be made highly accurate. Fortunately, where the waves are increasing in amplitude, an error in the correction makes little difference in the results. On the other hand where the amplitude is diminishing, the after-vibrations dominate, the correction is of the greatest importance, and any estimation of the forces acting yields a correspondingly inaccurate result.

The error in estimating the aortic cross section area, judged from the scattering of Suter's (1898) autopsy data (cf. Bazett, 1935), and Vaquez and Bordet's (1928) x-ray study, would have a relatively small effect on an estimate of the cardiac output in normal persons. But if pathological dilatation of the aorta went unrecognized the cardiac output would be seriously underestimated. X-ray examination will help to detect patients subject to this error and it might provide a basis for correction.

Two other sources of error should be kept in mind. If the direction of cardioaortic axis differs from the normal an error results. If blood regurgitates through the heart valves the result is related to the total stroke volume, and not to that part, estimated by the gas methods, which contributes to the circulation.

When the ballistic record has the normal form our results agree so well with those obtained by ethyl iodide that we see no reason for continuing the latter method, so time consuming and so difficult in comparison. When the ballistic record is abnormal, the accuracy of the estimation of cardiac output is more uncertain because we have secured so little data.

Patients with abnormal ballistocardiograms are often very sick and advancing this part of the field will be a slow process.

When the ballistocardiogram is used to compare the cardiac output of a single subject under different conditions most of its uncertainties disappear. For short experiments a continuous record of the cardiac output can be secured.

The shape of the ballistic waves indicates the curve of systolic blood velocity in the great vessels. Abnormal forms of this curve, similar to some of those we assumed (fig. 5), and changes in its shape under stress, are indicated by records of the volume of dogs' ventricles during systole, obtained in acute experiments by Piper, Patterson and Starling (1914) and by Wiggers and Katz (1922). Our method can identify similar changes in man.

Two small waves, not accounted for by the movement of blood in the great arteries, deserve mention. A wave in mid diastole is a constant feature of our records though often interfered with by the after vibrations. Coincident with the fall of the V wave of the venous pulse it is probably a record of impacts set up by blood filling the heart. The H wave begins at the onset of electrical systole and precedes ejection. This movement is usually headward, but at times it is preceded by a footward deflection. It is probably due to movements of the heart's center of mass during the pre-ejection phase of contraction. No effect of the auricular contraction can be certainly identified in normal records, but in heart block it shows clearly.

SUMMARY

Apparatus is described for recording the forces set up by the heart's recoil and the blood's impacts in man. The results obtained are characteristic of the subjects and easily reproduced.

When a subject lies on the table a damped vibration may be elicited which has a different frequency from that inherent in the apparatus. This vibration is well damped but not critically damped. The resulting errors have been evaluated by applying known impacts to the body and comparing these forces with the records obtained. From these results methods of avoiding or minimizing the errors have been devised.

In a theoretical study the form of the ballistic curve has been related to the curve of blood velocity in the great arteries during systole. Abnormal curves, encountered in disease, have been derived theoretically from abnormal blood velocity curves. The same abnormalities have been produced by asphyxiating or directly damaging the heart in animal experiments.

The relation between the ballistic waves and the cardiac output has been studied empirically by comparative experiments. Early in this

work we demonstrated that there was significant correlation between them. Later the theoretical study provided formulae for cardiac output in terms of certain aspects of the ballistic waves, the aortic cross section area, and the pulse rate. When these formulae were used great improvement in the correlation resulted, the cardiac output calculated from the ballistic waves showing satisfactory agreement with that estimated by the ethyl iodide method in 28 of 30 consecutive cases.

When the ballistic curve has a normal form, there is ample evidence that the cardiac output can be estimated from it with reasonable confidence. But, where the form of the ballistic record is abnormal, different formulae must be employed and, because such patients are hard to find and often very sick, evidence for the correctness of these formulae is still meagre.

The ballistocardiogram seems particularly suited for estimating changes in the cardiac output of single individuals.

From the point of view of both the subject and the operator this method is the simplest, easiest, and most rapid means of estimating the cardiac output that has been proposed. A purely mechanical device, it has been in daily use for three years without trouble of any kind.

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THE HEMODYNAMIC EFFECT OF THE DIOXANE DERIVATIVE 933 F ON TRAINED UNANESTHETIZED NORMAL AND RENAL HYPERTENSIVE DOGS AND ITS EFFECT ON THE PRESSOR ACTION OF RENIN[1]

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Little information on the hemodynamic action of drugs on the unanesthetized normotensive dog can be found, and none, as far as we could determine, on the unanesthetized hypertensive dog. This information would seem more applicable to the effects obtainable in man than data on anesthetized animals, since the possibility is removed that anesthesia might alter or mask the effect of the drug. Such studies require careful training of the animals used, and a painless method of determining the systemic blood pressure. Our experience with renal hypertension (1) has demonstrated that this is possible when the Hamilton needle manometer (2) is employed. Such trained animals we have found are disturbed very little when drugs are injected intravenously, and it appears, therefore, that the effects obtained are due to the drug action alone. The dogs can be trained so that they remain quiet on the board during the period of observation and show no signs of excitement as judged by restlessness, heart rate and blood pressure when saline solution is injected intravenously. No local anesthesia is necessary.

It occurred to us that if the chemical mediator responsible for the production of renal hypertension was sympatho-mimetic in character, then an agent which is known to reverse or diminish this pressor action should have a greater effect in the hypertensive than in the normal animal. Furthermore, if renin is related to renal hypertension, this agent should act on renin in a manner similar to that of its action upon the renal hypertensive dog. The dioxane derivative 933 F, piperidomethylbenzodioxane, is such an anti-sympathomimetic substance. It reverses the hypertension produced by adrenalin in the chloralosanized (3) and unanesthetized normal dog (4) and resembles the action of ergotamine in this respect (5, 6).

933 F was injected intravenously in trained normal and hypertensive

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unanesthetized animals in doses which reverse or lessen the pressor action of adrenalin. This was found to be 1–5 mgm./kgm. The effects of 933 F on the normal and hypertensive dogs were compared, as were the effects of renin on these animals before and after 933 F.

METHODS. Trained dogs were used. The hypertensives had had elevated pressures for periods varying from two weeks to several months previous to the experiments. The normotensive dogs were carefully selected and were trained (as described previously (1)) for a period of

TABLE 1

Effect of the dioxane derivative, 933 F, on the normal and renal hypertensive dog's blood pressure and heart rate

NORMOTENSIVE DOGS							HYPERTENSIVE DOGS						
1	2	3	4	5	6		1	2	3	4	5	6	
Dog no.	Control pressure	Heart rate	Minimum pressure after 933 F	Heart rate	Pressure fall (col. 2-4)		Dog no.	Control pressure	Heart rate	Minimum pressure after 933 F	Heart rate	Pressure fall (col. 2-4)	
					Systolic	Diastolic						Systolic	Diastolic
1 mgm./kgm.													
	mm. Hg	*beats per minute*	*mm. Hg*	*beats per minute*	*mm. Hg*	*mm. Hg*		*mm. Hg*	*beats per minute*	*mm. Hg*	*beats per minute*	*mm. Hg*	*mm. Hg*
U-94	175/100	132	175/100	240	0	0	U-31	215/130	168	180/110	336	35	20
V-43	150/75	156	135/70	252	15	5	U-84	230/135	132	220/130	288	10	5
3 mgm./kgm.													
U-54	140/80	84	130/80	144	10	0	U-39	185/105	96	175/90	312	10	15
U-99	175/85	108	115/65	204	60	20	U-45	225/100	96	150/50	216	75	50
U-94	150/75	156	85/50	216	65	25	U-81	220/135	132	235/130	156	15*	5
U-54	130/85	96	110/65	156	20	20							
Average					39	22						23	23
5 mgm./kgm.													
U-91	165/90	108	105/70	264	60	20	U-31	235/120	132	180/100	276	55	20

* Rise.

several weeks until successive readings showed a diastolic variation of ±5 mm. Hg or less. The hypertensive dogs were already trained. However, the diastolic pressure shows a greater variation on successive readings in the hypertensive than in the normotensive trained dog (±10 mm. Hg). The general reaction to the drugs were noted, and the systolic, diastolic and pulse pressures and the heart rate were measured on the photographic records of the femoral arterial pressure. The drugs were injected during the blood pressure determinations in order to study the immediate effects. Following this, pressures were recorded at regular intervals until conditions had returned to or near normal.

The control adrenalin injection was made 1 to 3 hours before the injection of 933 F, and the control renin injection was made 1 to 4 hours before the injection of 933 F. The second injection of adrenalin and renin was made within a maximum of 10 minutes after the 933 F injection was completed in order to be certain that the 933 F effects were still present. The interval of 1 to 4 hours between renin injections is sufficient we

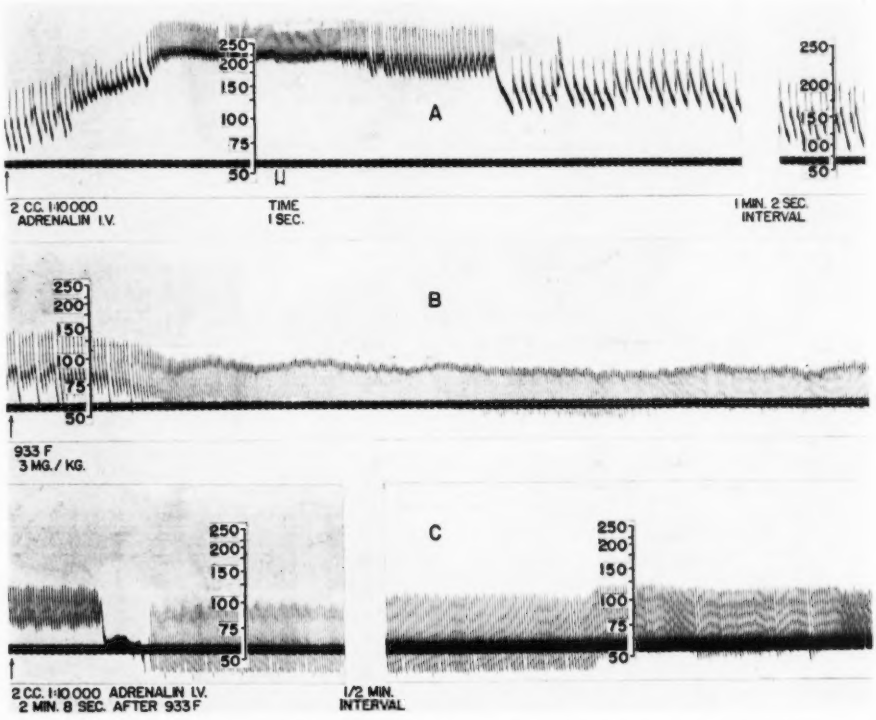

Fig. 1. Typical record of femoral arterial blood pressure in a trained and unanesthetized normotensive dog showing the effect of intravenous 933 F (curve B) and intravenous adrenalin before (curve A) and after 933 F (curve C). The calibrations are indicated in millimeters of mercury on each record. The doses used and the time of injection are indicated beneath each curve.

believe to avoid the refractory period which is said (7 and 8) to follow renin injection.

Discussion of results. (*a*) *The action of 933 F on normal and hypertensive dogs.* Our results showing blood pressure and heart rate changes are shown in table 1 and typical experiments are illustrated in figures 1 and 2, B and C. In brief, we found:

1. In all instances in both normal and hypertensive animals, the injection of 933 F in doses from 1–5 mgm./kgm. caused a marked fall in diastolic and pulse pressure which lasted about 15 minutes. In addition, there occurred an extreme tachycardia persisting longer than the blood pressure drop, and lasting as long as $\frac{3}{4}$ to 1 hour.

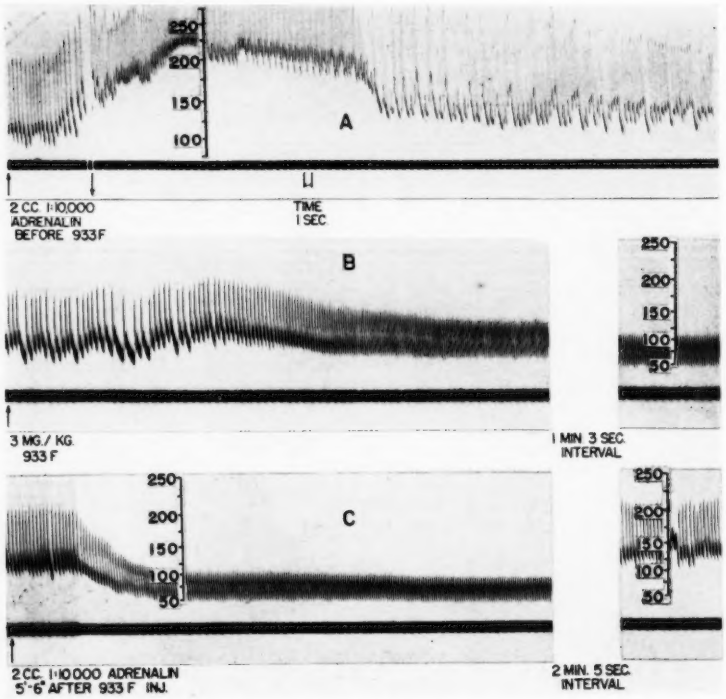

Fig. 2. Typical record of femoral arterial blood pressure in a trained and unanesthetized hypertensive dog showing the effect of intravenous 933 F (curve B) and intravenous adrenalin before (curve A) and after 933 F (curve C). Calibrations are in millimeters of mercury; dosages used and time of injection are indicated beneath each curve.

2. The injection of 933 F in doses of 1–5 mgm./kgm. caused a tachycardia, profuse salivation, pupillary dilatation and shivering. This is in accord with Handovsky (4) who reported these effects using larger doses (10 mgm./kgm.).

3. No constant difference was noted in the action of 933 F in normotensive and hypertensive dogs. The depressor action was of the same

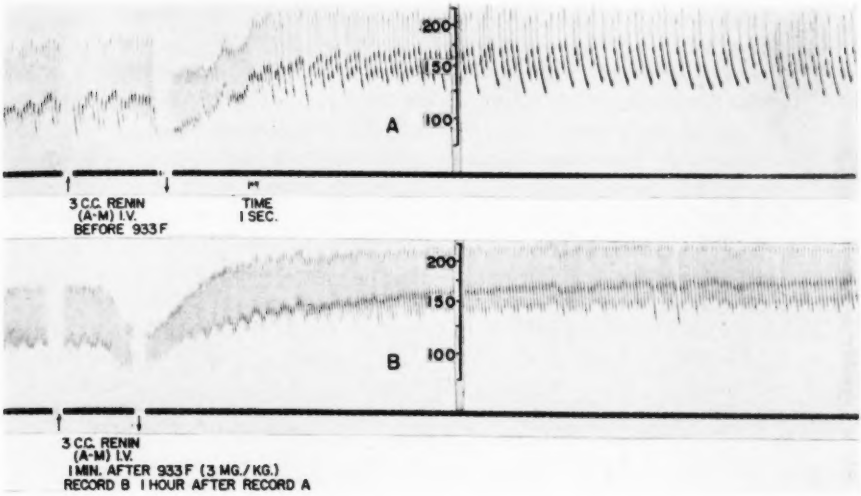

Fig. 3. Typical record of femoral arterial pressure in a trained unanesthetized normotensive dog showing the effect of intravenous renin before (curve A) and after 933 F (curve B). Doses and time of injection are indicated beneath each curve.

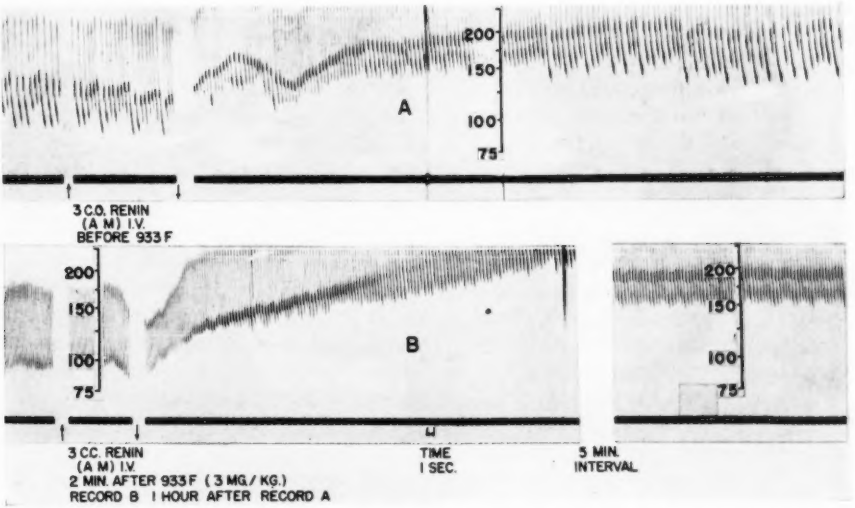

Fig. 4. Typical record of femoral arterial pressure in a trained unanesthetized hypertensive dog showing the effect of intravenous renin before (curve A) and after (curve B) 933 F. Doses used and time of injection are indicated beneath each curve.

magnitude and duration in both types of animals (cf. figs. 1 and 2 and table 1).

4. 933 F reversed or lessened the pressor action of adrenalin (2 cc. of 1/10,000) in both normotensive and hypertensive dogs (cf. figs. 1 C and 2 C), and no differentiation in the magnitude of this effect could be established in the two types of animals. The increase and decrease in pulse pressure seen in figures 1 and 2 C occurred in both.

5. With tachycardia, the phasic sinus arrhythmia disappeared. This in turn led to less respiratory fluctuation in blood pressure.

These results indicate that:

1. The abolition of the vaso-constrictor action of sympatho-mimetic drugs like adrenalin by 933 F is operative in unanesthetized animals.

2. The chemical mediator of renal hypertension is not sympatho-mimetic in nature like adrenalin. Otherwise 933 F should have caused a greater drop in blood pressure in the hypertensive than in the normal dog.

3. The unpleasant side actions of 933 F in dosages of 1–5 mgm./kgm. preclude its possible use as a depressor drug in clinical hypertension.

(*b*) *The effect of 933 F on the pressor action of renin in normal and hypertensive dogs.* Four different preparations of renin obtained from normal dog, hog, rabbit and pig kidneys made by slightly different methods were used[2]. The results of injection of these extracts is shown in table 2, and typical experiments are illustrated in figures 3 and 4. Our results show that:

1. Renin had a quantitatively similar action in intensity and duration on the normotensive and hypertensive unanesthetized dog.

2. The effects of the various renins used were similar.

3. In some instances a fleeting tachycardia and depressor action were seen, presumably due to impurities in the preparation. No unpleasant side reactions were noted. Accompanying the tachycardia, the phasic sinus arrhythmia lessened or disappeared.

4. 933 F did not minimize, reverse or shorten the pressor action of renin, although on several occasions it appeared to exaggerate the preliminary depressor action.

These results indicate that renin, as typified by these preparations, is not sympatho-mimetic in nature, otherwise 933 F should have reversed or lessened its pressor action.

This conclusion, therefore, is not in accord with that of Williams (9) who reported that in barbitalized rats, ergotamine inhibited the pressor

[2] We wish to acknowledge our indebtedness to Drs. Louis Leiter of the University of Chicago, A. C. Ivy of Northwestern University, G. Wakerlin of the University of Illinois and D. I. Abramson and I. A. Mirsky of the May Institute for Medical Research, Jewish Hospital, Cincinnati through whose kindness the four samples of renin were obtained.

action of renin. The exaggeration of the early depressor action which we noted, might support Williams' (10) observation that in addition to the heat-labile, alcohol insoluble, non-dialyzable substance in kidney extracts which resembles Tigerstedt's (11) renin, there is another substance, adrenalin-like in character, which is obtained by perfusing freshly removed kidneys. This substance Williams called "perfusin," and its pressor action he found was inhibited by ergotamine. Our results tend to minimize the significance of perfusin, since if renal hypertension were due to its constant release from the kidney as Williams suggests, 933 F should have reduced the hypertensive action of renin. This did not occur.

TABLE 2

Effect of renin on blood pressure in normal and renal hypertensive dogs and after dioxane derivative, 933 F (3 mgm./kgm.)

NORMOTENSIVE DOGS					HYPERTENSIVE DOGS				
Dog no.	Maximum pressure rise with renin				Dog no.	Maximum pressure rise with renin			
	Before 933 F		After 933 F			Before 933 F		After 933 F	
	Systolic	Diastolic	Systolic	Diastolic		Systolic	Diastolic	Systolic	Diastolic
Renin no. 1 (Leiter)									
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
U-94	65	55	75	50	U-45	50+*	65	95	90
Renin no. 2 (Ivy)									
U-94	55	50	50	45	U-45	50+*	60	55	70
Renin no. 3 (Wakerlin)									
U-94	25	25	0	20	U-45	10	25	0	30
Renin no. 4 (Abramson and Mirsky)									
U-94	55	40	50	45	U-45	30+*	65	40+*	60

* Pressure record went off film.

The demonstration that neither the action of the chemical mediator of renal hypertension nor the pressor action of renin is reversed or lessened by 933 F shows that the chemical mediator and renin share one common characteristic, namely, neither is sympatho-mimetic in action. It does not follow from this, however, that renin necessarily is the pressor substance involved in renal hypertension. If renin represents the chemical mediator, some reasonable explanation must be given to explain the absence of "refractoriness" to renin injection in experimental renal hypertension such as occurs when injections of renin are repeated in rapid suc-

cession in normal dogs (7, 8). It has been suggested that this refractoriness is due to impurities and not to the renin (12).

SUMMARY

1. A method of studying the hemodynamic action of drugs in trained unanesthetized dogs is described. This method was found to cause no discomfort nor any significant hemodynamic change when saline solution in the quantities used to dissolve the drugs was injected intravenously.

2. The dioxane derivative 933 F in doses of 1 to 5 mgm./kgm. produces a reversal or inhibition of the adrenalin pressor action in the trained unanesthetized dog.

3. Intravenous injection of 933 F produces a quantitatively similar depressor action in the normal and hypertensive unanesthetized dog. It would appear, therefore, that the chemical mediator of experimental renal hypertension is not sympatho-mimetic in action.

4. The pressor material, renin, obtained from kidney extracts, has a quantitatively similar action in normal and hypertensive dogs.

5. This pressor effect is not inhibited, reversed or abolished by 933 F. Renin, therefore, does not appear to act as a sympatho-mimetic substance.

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ALTERATIONS IN DARK ADAPTATION UNDER REDUCED OXYGEN TENSIONS

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Since the original observations of Paul Bert and the early balloonists a number of experiments have demonstrated that a diminished partial pressure of O_2, as encountered at high altitudes, gives rise to marked alterations in the functioning of the central nervous system (McFarland, 1932). The nervous tissue appears to be particularly susceptible to a deficit of O_2. The experiments of Heymans and his associates (1933), for example, have shown that the changes in the cerebrum are apparently irreversible if acute anoxia is continued longer than 5 to 8 minutes, while the same is true of lower centers and the spinal cord after 20 to 30 minutes. Sensory functions, as well as complex behavior like memory, association and judgment, which are mediated by cortical neurones, are severely impaired by variations in the partial pressure of O_2 and CO_2. Light sensitivity might be expected to show marked effects under anoxia, since the retina is anatomically a part of the brain. The metabolism of the retina appears to be similar to that of the brain, as judged by experiments on excised tissue (Warburg, 1927).

Under moderate degrees of anoxia, the effect on vision is manifested not in the blurring of outlines, but in a general darkening of the visual field. Under acute anoxia there may be an intermittent cessation of all visual experiences, even previous to loss of consciousness. Pilots have frequently reported a general darkening of the visual field while flying at great heights without O_2 (18,000 ft. and above) (McFarland, 1932). In Douglas bag experiments in the laboratory, we have noticed dimming of the lights during sudden exposure to partial pressures of O_2 simulating 12,000 to 14,000 feet and a marked increase in the brightness of lights on being quickly changed back to room air. In experiments with an optical wedge Wilmer and Berens (1918) found an impairment in light sensitivity

[1] The authors wish to express their indebtedness to Mr. Meyer H. Halperin for assistance in giving the tests and to The Linde Air Products Company for the generous supplies of nitrogen used in these experiments.

in low pressures. During trans-Pacific flights at 11,000 to 12,000 feet altitude, McFarland and Edwards (1937) observed a decrease in brightness sensitivity in pilots with a similar apparatus. Schubert (1933) and Gellhorn (1936) have both observed a considerable decrease in visual intensity discrimination under acute anoxia with the use of Masson discs. The effects of anoxia on dark adaptation have been studied by Fischer and Jongbloed (1935–36) in low pressures and by Bunge (1936–37) with a pulmotor apparatus, where the rate and depth of breathing were kept constant. Both reported significant changes, which the former attributed to an interference with the regeneration of the photosensitive substances in the retina and the latter to the effect on the nervous tissue of the brain and retina.

Certain other aspects of the visual mechanism have been studied under low O_2. In testing pilots for high altitude during the World War in low pressures, Wilmer and Berens (1918) found a constriction of the visual fields, and diminished visual acuity. They reported that there were no significant effects at simulated altitudes of 10,000 feet, only slight deficiencies in certain visual functions at 15,000 feet, while at 20,000 feet or over the impairment was very marked. A lengthening of the latent period of the negative after-images was observed by Gellhorn and Spiesman (1935) under acute O_2 want. At times they noticed that there was a complete absence of any after-image. In subjects acclimatized to high altitudes on the Chilean Expedition, McFarland (1937) found variations in the negative after-images at 17,500 feet and at 20,000 feet. By means of the technique of angioscotometry, Evans and McFarland (1938) observed variations in sensitivity in the central visual field. With progressive O_2 deprivation, the angioscotoma (projected defect related to the retinal perivascular spaces) widened progressively until it obliterated the visual field except for a region 8 to 10 degrees about the macula. They attributed these alterations not to the changes in size of the retinal vessels, nor to edema of the retina, but to the dysfunctioning of the synapses in the retina due to the lack of O_2.

In the experiment described below, we have measured the phenomena of the darkening of the visual field under anoxia by the usual procedures of studying night blindness, i.e., the measurement of the return of the capacity to see in the dark following exposure to a light of high intensity. Interest in the study centered not only in the measurement of a change in subjective experience in relation to a known degree of O_2 deprivation but also in attempting to determine the extent to which such a sensory process might be attributed to central (cerebral) or to peripheral (ocular) factors. We were also interested in whether the effects of the anoxia would be manifested in the photochemical processes in the retina with associated changes in the characteristics of the dark adaptation curves, or whether the phenomena

could be attributed directly to the neurones and synapses. In addition, this test promised to be a sensitive one for indicating the initial effects of anoxia on the nervous tissue. The practical implications are obvious in the field of aviation in case it was determined that a significant amount of night blindness resulted from the diminished tensions of O_2 at moderate or at high altitudes.

EXPERIMENTAL PROCEDURE. The thresholds of light sensitivity were measured with a "biophotometer" (Jeans et al., 1937). In this apparatus the source of the light adaptation is a rectangular ground glass screen (10 x 13 cm.), centrally fixated about 32 cm. from the subject's eyes. This screen was illuminated by a 100 watt lamp. The amount of current was controlled by a rheostat and voltmeter, so that the brightness of the screen was 100 millilamberts (106 footcandles, as measured by a Macbeth illuminometer).

Certain recognized inadequacies of the biophotometer were overcome for these comparative studies by using an altered technique from that recommended by the designers. Following the initial light adaptation, the method of measuring the subsequent thresholds in darkness was as follows: a metal screen, 24 cm. from the subject's eyes, containing 5 circular holes in the form of a quincunx (as on dice), concealed the above-mentioned ground glass. The holes of the quincunx contained filters so that the intensity of the transmitted light decreased from left to right. (In our procedure the dimmest 2 lights at the right were not used.) This screen was illuminated by a dim, variable source of light which was controlled by a rheostat, the handle of which turned over a dial numbered from 0 to 80. The voltage supplied to this lamp at an arbitrary dial reading was controlled by a second rheostat and a voltmeter. A calibration table enabled one to convert the dial readings into millifootcandles of brightness of the center dot of the quincunx, which was used as the measuring light. The diameter of this dot of light subtended an angle of 70 minutes of arc from the point of observation. It fell on the 135° meridian of the superior nasal quadrant of the retina of the right eye and on a corresponding point of the left retina. The upper left-hand dot of the quincunx, which was 6° from the middle dot and always brighter, was used as a fixation point. All of the observations were binocular.

TECHNIQUE. Before measuring the thresholds, and between successive determinations, the intensity of the measuring light was kept at its minimum, so that none of the dots were visible (or at most, during the latter part of dark adaptation, only the brighter left-hand dots could be seen). To make a reading, the intensity of the light was gradually increased and as soon as the subject could see the left-hand dots, he responded and fixated the upper one of these. When the center dot became barely visible, the subject again responded. The dial reading at this point was recorded and

the light immediately turned down to its minimum intensity, at which it remained until the next reading was made. The rate at which the intensity was increased was intentionally varied so that no time cues could be used by the subject. It was found in all cases that the readings were entirely independent of the time taken to reach the threshold, which varied from 3 to 10 seconds.

No attempt was made to keep the observer's pupil constant by using an artificial pupil. Hecht and Schlaer (1938) state that this is unnecessary, except under special conditions, since the change of the pupil from the light-adapted condition to the dark-adapted condition happens so quickly that it hardly enters into the measurement. Under conditions of O_2 want, however, it is possible that significant variations in pupil size may occur, due to stimulation of the sympathico-adrenal system. In previous studies of the effects of anoxia on visual functions, it has been observed that the variations in pupil size were too small to be detected with a pupillometer (Bunge, 1936-37; Evans and McFarland, 1938).

The tests were given in a Barach oxygen chamber (7 x 8 x 8 ft.), in which the gases, temperature, ventilation and humidity could be regulated. The concentration of O_2 was reduced to the desired percentage by admitting nitrogen from cylinders outside the chamber. The accumulation of CO_2 rarely exceeded 0.7 per cent (cf. table 3). Samples of the air were taken at intervals from outside the chamber and analyzed on the Haldane apparatus. The ventilation was provided by a motor blower unit. The air current was passed through a tank which contained ice to cool and dry the air. The temperature was maintained between 65° and 70°F. and the humidity between 40 per cent and 50 per cent. The experimenter was supplied with O_2 through a nasal catheter from a cylinder inside the chamber.

The 20 *subjects* were graduate students, medical students and doctors of medicine. The age range was from 20 to 30 years, with the exception of Subject 2, who was 42. Two were female (no. 7 and no. 10), the others male. All were free of organic disease and of ocular defects other than correctible refractive errors. Complete eye examinations were given to all the subjects by Dr. Charles Rosenthal. The subjects were non-athletic in type and, on the average, in poor physical condition.

Each subject went through a 2-hour practice period, during which time the dark adaptation test was given repeatedly. This was sufficient so that each subject's report was very consistent. During this initial testing period, practice was also given in the methods used to collect the samples of alveolar air. These air samples were taken in duplicate during the control session (sea level) and at each of the simulated altitudes by the Haldane expiratory method, stored in Bailey sample bottles and analyzed subsequently on the Haldane apparatus.

After the subject entered the chamber, 2 control series in air were given, followed by tests at 15.7 per cent O_2 (7,400 ft.), 13.7 per cent O_2 (11,000 ft.) and 11.7 per cent O_2 (15,000 ft.). The subject was permitted to dark adapt for at least 15 minutes before the test was made, during which time a few preliminary readings were taken. (From this time to the end of the experiment the subject could see no light other than that in the apparatus.) He was then exposed to the adapting light for 3 minutes (measured with a stopwatch). During this time his pulse rate and blood pressure were recorded during the light-adapting period and at intervals thereafter, so as to follow the condition of the subject. The blood pressure determinations were made with a Tycos recording sphygmomanometer. Toward the end of dark adaptation, alveolar air samples were taken in duplicate, 5 minutes apart. An air sample of the atmosphere in the chamber was also taken, midway between the 2 alveolar samples. The total amount of time spent at each altitude was about 35 minutes. The ascent from the first to the second altitude (11,000 ft.) required 5 minutes, and that to the third altitude (15,000 ft.) 6 minutes. The entire experiment lasted approximately 3 hours.

At the end of the test at 15,000 feet a mask supplying 7 liters of pure O_2 per minute was placed over the mouth and nose of the subject and the thresholds were measured every 30 seconds thereafter until a constant threshold was reached. This required 5 minutes at most; frequently as little as 2 minutes was sufficient. The chamber was then opened and a fan turned on so as to blow out the high nitrogen mixture from the chamber. The subject was allowed to rest for 15 minutes, and the dark adaptation test was repeated. In all cases the curve was as close to the 2 initial controls as these were to each other.

Results. The average light intensity thresholds for the 20 subjects during 20 minutes of dark adaptation at sea level and under the various conditions of O_2 deprivation as indicated above are shown in table 1. The points on the dark adaptation curves were computed by averaging the thresholds of all the subjects at corresponding times. Composite curves, based upon the combined data of table 1, are presented in figure 1.

Upon inhaling O_2, as shown in table 1, the threshold returned to the normal level within 2 to 3 minutes. In 4 instances the subjects, while still dark adapted, were again deprived of O_2 and the threshold rose within 2 minutes to the 15,000 foot level. Restoration of the O_2 supply again resulted in a sudden recovery of sensitivity. This parallelism of threshold (or sensitivity) to pO_2 without any exposure to light indicates that the photochemical system—or at least the *regeneration* of the visual pigment—could not have been involved.

The threshold measurements were recorded in terms of "dial units" on the apparatus. A calibration table giving the equivalent value of each

dial unit in terms of millifootcandles is supplied by the manufacturers. Upon plotting dial units against the logarithm of intensity, it was found that a precisely linear relationship existed, the equation for which was $\log I = 0.67 - \frac{R}{24}$ (where I = intensity in millifootcandles and R = dial reading). Hence we could handle our data in terms of the dial units, and

TABLE 1

Average light intensity thresholds for 20 subjects during 20 minutes of dark adaptation at the simulated altitudes as indicated

TIME	CONTROL	ALTITUDE I, 7,400 FEET	ALTITUDE II, 11,000 FEET	ALTITUDE III, 15,100 FEET	RECOVERY WITH OXYGEN
	dial units	*dial units*	*dial units*	*dial units*	
20 sec.	21.3	20.7	18.7	17.6	
½ min.					63.6
1 min.	31.5	30.8	28.6	26.0	67.5
1½ min.	34.9	34.2	31.8	30.6	67.9
2 min.	38.1	36.6	35.0	32.5	69.0
2½ min.	41.2	39.0	36.6	34.1	
3 min.	43.0	41.0	38.0	35.8	70.4
3½ min.	45.1	42.2	39.8	37.2	
4 min.	46.7	43.3	41.8	39.2	71.8
4½ min.	49.2	45.8	43.1	40.2	
5 min.	50.9	48.5	45.0	41.9	71.8
6 min.	55.0	51.9	49.3	46.0	
7 min.	58.1	55.2	52.5	48.2	
8 min.	60.5	58.0	55.6	51.6	
9 min.	62.8	61.0	56.7	53.7	
10 min.	65.0	62.8	59.2	54.9	
11 min.	66.5	63.3	60.6	55.9	
12 min.	67.4	65.6	61.6	57.0	
13 min.	68.4	66.4	63.2	57.6	
14 min.	69.0	67.0	63.9	58.2	
15 min.	69.4	67.7	63.6	58.3	
16 min.	70.2	68.0	63.9	59.1	
17 min.	70.7	68.6	64.9	59.1	
18 min.	71.0	68.5	65.2	59.5	
19 min.	71.2	68.8	65.2	59.0	
20 min.	71.4	68.7	65.3	58.9	

convert to log I units at the end, if desired. The dark adaptation curves plotted against dial units are of precisely the same shape as when plotted against log I. In the case of threshold differences, the value in dial units is converted to logarithmic units simply by dividing by -24; a decrease of 24 dial units is equivalent to an increase of 1 log unit, or a rise of 10× in intensity.

The extent of the changes in each subject and the statistical reliability

were estimated as follows: The dial readings at sea level and at each altitude were listed in parallel columns. The differences were then computed between the thresholds at each altitude and those at the corresponding time at sea level. These differences were averaged, and this was then converted to log units. The antilog of this value thus represents the average ratio of the threshold at the given altitude to the threshold at the corresponding time at sea level. The results for each subject at the 3 simulated altitudes compared with sea level (control) are shown in table 2. The statistical reliability of these differences was computed by Fisher's (1932) method for the significance of unique samples. As shown in table 2, the differences

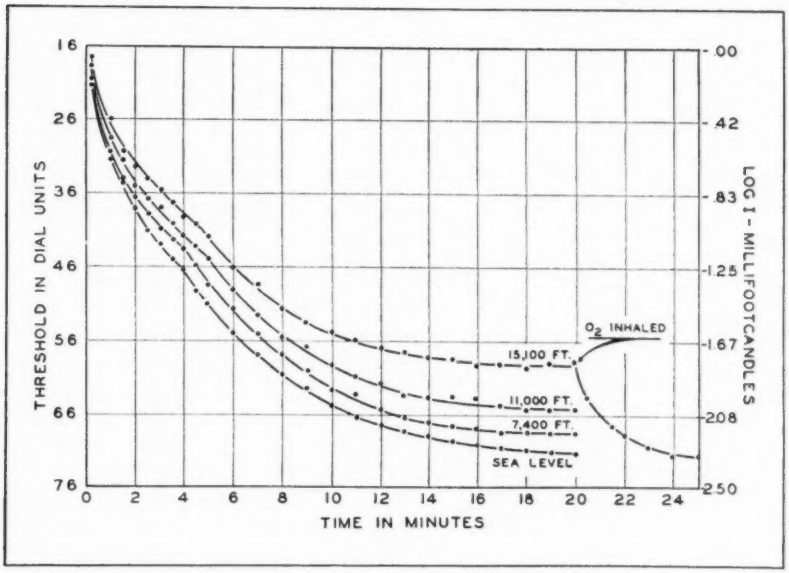

Fig. 1

between the thresholds at sea level compared with 7,400 feet were not significant in the case of 5 subjects. At 11,000 feet the elevation in the threshold was significant for all but 1 subject, and at 15,000 feet the differences were highly significant for all subjects.

The group averages were obtained from the data on each subject, and the group reliability determined by the same method. These averages are an estimate of the extent of the effect of O_2 deprivation on the light sensitivity of the subjects during 20 minutes of dark adaptation following a 3-minute exposure to an intensity of 100 millilamberts. They are plotted against altitude in figure 2. The curve is almost precisely parabolic, the

effect increasing as the square of the altitude. They are also shown in relation to alveolar O_2 tension in figure 2.

TABLE 2

Average difference between thresholds at each altitude and corresponding thresholds at sea level, during 20 minutes of dark adaptation

Statistical significances of these differences given, as determined by Fisher's method (1932).

SUBJECT NO.	ALTITUDE I—7,400 FEET		ALTITUDE II—11,000 FEET		ALTITUDE III—15,075 FEET	
	Average elevation in threshold compared with sea level	P*	Average elevation in threshold compared with sea level	P*	Average elevation in threshold compared with sea level	P*
	log. units		*log. units*		*log. units*	
1	0.104	<0.01	0.310	<0.01	0.506	<0.01
2			0.458	<0.01	0.618	<0.01
3	0.021	0.06	0.254	<0.01	0.401	<0.01
4	0.095	<0.01	0.250	<0.01	0.326	<0.01
5	0.091	<0.01	0.142	<0.01	0.337	<0.01
6			0.000	1.00	0.305	<0.01
7	0.228	<0.01	Collapsed			
8	0.060	<0.01	0.142	<0.01		
9	0.000	1.00	0.073	<0.01	0.231	<0.01
10	0.184	<0.01	0.237	<0.01	0.309	<0.01
11	0.229	<0.01	0.341	<0.01	0.522	<0.01
12	0.112	<0.01	0.349	<0.01	0.422	<0.01
13	0.023	0.53	0.371	<0.01	0.476	<0.01
14	0.182	<0.01	0.283	<0.01	0.470	<0.01
15	0.016	0.25	0.124	<0.01	0.126	<0.01
16	0.073	<0.01	0.086	<0.01	0.186	<0.01
17	0.155	<0.01	0.268	<0.01	0.423	<0.01
18	0.083	<0.01	0.133	<0.01	0.509	<0.01
19	−0.007	0.98	0.167	<0.01	0.365	<0.01
20	0.202	<0.01	0.271	<0.01	0.615	<0.01
Average	0.1028	Five not significant	0.2242	One not significant	0.3971	All highly significant
S. D.	0.0759		0.1145		0.1333	
C. R.	5.58		8.31		12.28	
P*	<0.01		<0.01		<0.01	

* Values of P less than 0.05 indicate significant differences.

The correlations between the effect at one altitude and at each other altitude were computed for the group by the rank difference method. The

correlations were as follows: altitude I (7,400 ft.) and altitude II (11,000 ft.) = 0.76 ± 0.07; altitudes I and III (15,000 ft.) = 0.71 ± 0.08; altitudes II and III = 0.88 ± 0.03. These high correlations indicate that each subject tended to maintain his relative position within the group, i.e., the subject who showed a marked increase in threshold at 7,400 feet reacted in a similar way at 11,000 and 15,000 feet in relation to the other subjects of the group.

The partial pressures of O_2 and CO_2 in the alveolar air in relation to the tensions of the O_2 and CO_2 in the chamber are shown in table 3. The

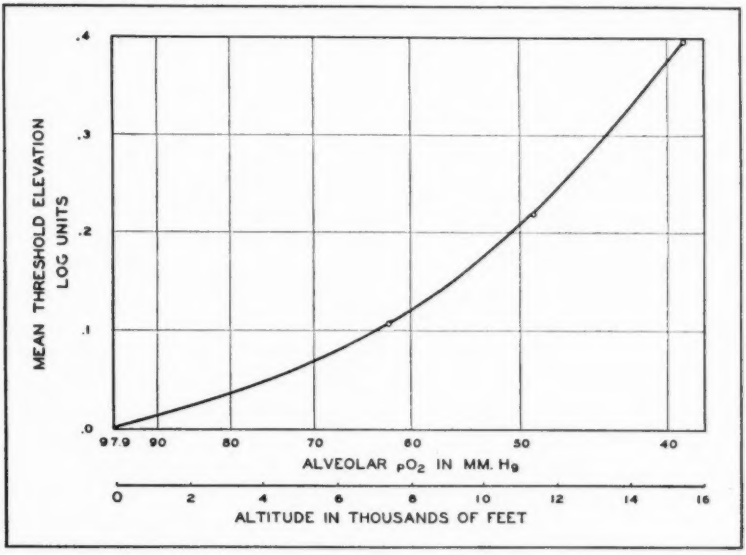

Fig. 2

partial pressure of O_2 in the alveolar air in relation to the mean threshold elevation in log units is shown in figure 2.

The variations in pulse rate and blood pressure were not marked, with the possible exception of those few subjects who acclimatized very poorly to the diminished tension of O_2. On the average, there was a tendency for the pulse rate and systolic blood pressure to increase during the initial stages of each experiment, followed by a return to more normal values toward the end. The mean pulse rates and blood pressure for the group of 2 subjects are shown in table 4.

Discussion. The dark adaptation curves (plotting threshold against time as in fig. 1) were elevated progressively with increased altitude or

TABLE 3

The partial pressure of the alveolar oxygen and carbon dioxide for each subject in relation to the partial pressure of the oxygen and carbon dioxide in the chamber at each of the three simulated altitudes as indicated

SUBJECT NO.	SEA LEVEL ALVEOLAR AIR		ALTITUDE I				ALTITUDE II				ALTITUDE III			
			Alveolar air		Chamber air		Alveolar air		Chamber air		Alveolar air		Chamber air	
	pCO_2	pO_2	pCO_2	pO_2	CO_2	O_2	pCO_2	pO_2	CO_2	O_2	pCO_2	pO_2	CO_2	O_2
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*
1	40.5	96.9	40.0	65.4	0.56	15.46	41.9	48.3	0.72	13.37	38.3	41.6	0.91	11.59
2									0.37	13.33			0.63	11.50
3	43.3	98.5	42.3	64.9	0.59	15.36	41.5	53.2	0.67	14.10	42.7	36.4	0.78	11.75
4			45.2	50.5	0.28	15.73	42.0	43.0	0.40	13.77	38.1	35.0	0.55	11.59
5	47.3	91.5	45.3	55.6	0.33	15.22	44.5	40.5	0.42	13.29	42.0	34.2	0.51	11.30
6	41.5	105.5					39.0		0.45	13.85	37.1		0.60	12.10
7					0.41	15.82								
8					0.27	15.78			0.64	13.75				
9	46.1	86.7	43.3	58.7	0.24	15.53	42.5	46.3	0.41	13.47	40.3	36.7	0.56	11.53
10					0.47	15.69			0.61	13.82			0.71	11.87
11	41.7	99.0	43.6	60.0	0.44	15.83	43.3	47.1	0.55	13.71	42.0	35.7	0.63	12.09
12	42.1	94.6	42.2	61.5	0.37	15.86	43.7	47.4	0.49	13.83	41.7	39.1	0.60	11.72
13	43.7	96.4	40.2	70.7	0.29	16.01	41.4	49.7	0.40	14.37*	42.5	38.4	0.46	12.05
14	36.6	109.2	36.4	75.1	0.26	15.90	35.4	56.3	0.50	13.95	35.5	46.2	0.61	12.06
15	40.0	99.1	40.0	67.8	0.25	15.68	38.5	56.0	0.35	14.08	35.4	46.3	0.49	12.04
16	44.3	98.0	45.4	59.4	0.30	15.90	42.9	46.0	0.54	13.85	41.5	36.5	0.66	12.20
17	46.8	94.7	46.4	62.2	0.30	15.86	45.2	47.0	0.43	13.69	43.2	36.7	0.51	11.61
18	38.5	102.2	37.7	68.9	0.30	16.51*	38.4	54.5	0.41	13.98	36.8	45.1	0.60	11.76
19					0.19	15.97			0.31	13.51			0.23	10.59*
20					0.37	15.76			0.51	13.69			0.61	10.90*
Average...	43.04	97.88	42.15	63.13	0.35	15.77†	41.44	48.87	0.48	13.76‡	39.79	39.07	0.59	11.68§
S. D.....	3.50	5.34	3.06	6.34			2.69	4.68			2.75	4.13		
C. R.....			−1.66	−25.7			−2.37	−42.0			−3.82	−44.7		
P.....			0.13	<0.01			0.04	<0.01			<0.01	<0.01		

* These altitudes, somewhat different from those chosen later for study, were used in preliminary experiments.

† Equivalent altitude, 7,400 feet.

‡ Equivalent altitude, 11,000 feet.

§ Equivalent altitude, 15,075 feet.

TABLE 4

The group means of the pulse rate and blood pressures for 20 subjects under the various conditions as indicated: (a) approximately 7 minutes after reaching the simulated altitude; and (b) after approximately 25 minutes.

	PULSE RATE		SYSTOLIC AND DIASTOLIC BLOOD PRESSURE	
	(a)	(b)	(a)	(b)
Sea level—Control.....	67		121/72	
15.7 per cent O_2 (7,400 feet)	69	68	121/72	121/73
13.7 per cent O_2 (11,000 feet)	69	68	123/73	121/74
11.7 per cent O_2 (15,000 feet)	75	74	121/72	123/74

diminished partial pressure of O_2. The results are consistent, and the curves obtained while breathing diminished partial pressures of O_2 were approximately parallel to the curve at sea level. Hecht (1938) and others have shown that dark adaptation proceeds in 2 stages. The first step is rapid and is over in a few minutes, recording the behavior of the cones. The second is late in starting and goes on for half an hour or more. This delayed and slower section of the curve records the function of the rods and according to Hecht represents the regeneration of visual purple. Accepting this interpretation of Hecht, the dark adaptation curves shown in figure 1 suggest that the behavior of the cones was primarily involved during the first 4 to 5 minutes and the rods from 5 to 20 minutes. The anoxia apparently affected both the cone and rod sections of the curve, but more particularly the rod portion.

It seems reasonable to assume under the conditions of this experiment that thresholds relating to dark adaptation offer a sensitive test of the early effects of anoxia at altitudes where other sensory and mental tests given under similar conditions have failed to show reliable changes. At a simulated altitude of 7,400 feet, for example, there was an average decrease in sensitivity of 0.102 of a log unit, statistically significant changes being observed in all but 5 subjects. At 11,000 feet only 1 subject was not reliably affected, and the average decrease in sensitivity was 0.224 of a log unit. At 15,000 feet the decrease was 0.397 of a log unit, being statistically reliable for all subjects. The assumption that these changes are related to O_2 lack is clearly supported by their being suddenly and completely abolished by breathing O_2, as shown in figure 1.

These effects of anoxia on dark adaptation may probably be attributed to the conductive nervous tissue of the visual mechanism and to the connecting pathways from the retina to the cerebral cortex rather than to the alteration of the photochemical substances of the receptor cells of the retina for the following reasons. In the first place, the characteristics of the curves at sea level and at high altitude are strikingly similar in shape, both for the cone and rod portions, when they are superimposed one upon the other. Any impairing effect on the regeneration of visual purple might tend to change the characteristics of the curves, especially the rod portion. In the second place, the effects are largely abolished within approximately 2 minutes after the inhalation of excess concentrations of O_2 (cf. fig. 1). It is very unlikely that a regeneration of photochemical substances in the retina could take place so rapidly. The results of Wald et al. (1938) with a human subject on a vitamin A deficient diet suggest that at least 7 minutes must elapse before the intramuscular injection of large amounts of carotene affects visual adaptation, or that 20 minutes must pass before it reaches the normal rod level. In the third place, Bunge (1936–37) has shown that even following complete dark adaptation at sea level (45

to 60 minutes, during which time the regeneration of visual purple should be complete) there was a marked drop in sensitivity in darkness when subjects were exposed to acute anoxia (8 per cent to 10 per cent O_2). In this experiment we were also able to vary the threshold within 1 to 2 minutes in completely dark-adapted subjects by increasing or decreasing the partial pressure of O_2. These findings suggest that O_2 deprivation bears no direct relationship to the regeneration of the photochemical substances of the retina having to do with dark adaptation.

It is well known that psychic disturbances occur under acute anoxia and that therefore the decreased sensitivity might possibly be attributed to faulty responses due to alterations in consciousness. However, the responses of the subjects were too prompt and consistent for this to be the case, and the decreased sensitivity was apparent previous to any of the usual symptoms of altitude sickness, such as headache, nausea, sleepiness and indifference to the task at hand. In fact, most of the subjects were unaware of any alteration in the gases in the chamber until the higher simulated altitudes were reached. Also, the alterations in circulation as indicated in the pulse rate and blood pressure records of table 4 were too small to account for the lowered sensitivity.

The results obtained in this experiment may be directly attributed to the effects of anoxia on the nervous tissue. The cortical tissue appears to be extremely sensitive to lowered tensions of O_2 and to variations in the CO_2 which plays such an important rôle in the utilization of O_2 in the nerve cells. It is not surprising that retinal functions are very sensitive to a deficit of O_2, since Weinstein (1932) has shown that the consumption of O_2 and the production of CO_2 in the brain and retina are similar both aerobically and anaerobically. According to Warburg (1927) the retina has the greatest rate of respiration and of anaerobic glycolysis of any tissue studied.

There is no evidence that the effect of anoxia on dark adaptation is neither exclusively peripheral (retinal) nor exclusively central (cortical). No doubt both of these neural aspects of vision are involved. In comparing the dark adaptation curves, using monocular as contrasted with binocular observation, Clamann (1938) observed that they were not similar since the "summation," or extra-retinal effects, is involved in the latter. Since anoxia appeared to affect the monocular and binocular thresholds in different ways, he concluded that the effect must operate to some extent on the extra-retinal system. In a previous experiment using the technique of angioscotometry, on the other hand, we observed that the effects of anoxia on the central visual field were primarily retinal (Evans and McFarland, 1938). The angioscotoma (projected defect related to the retinal perivascular spaces) progressively widened under increasing O_2 lack until it obliterated the visual field except for a region 8 to 10 degrees about the

macula. These effects were attributed to the dysfunctioning of the retinal nervous tissue primarily at the synapses. Light sensitivity might be expected to vary under anoxia for somewhat similar reasons. It appears, therefore, from the evidence thus far available, that the effects of anoxia on dark adaptation depend upon both retinal and extra-retinal processes.

SUMMARY

1. The dark adaptation of 20 human subjects was measured in a low O_2 chamber where the partial pressure of O_2 could be reduced by dilution with nitrogen. The effect of O_2 deprivation on light sensitivity was followed for 20 minutes after a 3-minute exposure to a light of 100 millilamberts. Each subject was tested at a simulated altitude of 7,400 feet (15.8 per cent O_2), 11,000 feet (13.7 per cent O_2) and 15,000 feet (11.7 per cent O_2). The CO_2 was kept below 0.6 per cent. Following 2 control tests in air, each subject was held at the simulated altitude for approximately 35 minutes, 1 period immediately following the other. The final thresholds were obtained while inhaling O_2 at the end of each experiment.

2. The dark adaptation curves (plotting threshold against time) were progressively elevated with increasing O_2 deprivation (cf. fig. 1). These effects were counteracted within 2 to 3 minutes by inhaling O_2. The diminution in light sensitivity was statistically significant for all but 5 of the 18 subjects at the first simulated altitude of 7,400 feet, for all but 1 at 11,000 feet and for the entire group at 15,000 feet. The magnitude of these changes was 0.10, 0.22 and 0.40 of a log unit respectively.

3. The dark adaptation curves obtained under the various stages of anoxia were approximately parallel to the control curves obtained in air. The portions of the curves representing the behavior of both the cones and the rods were affected, especially the latter.

4. Samples of alveolar air were obtained at various intervals during the experiment. In plotting the alveolar partial pressure of O_2 and altitude in relation to decreased light sensitivity, as shown in figure 2, the curve was almost precisely parabolic, the effect increasing as the square of the altitude. The alterations in pulse rate and blood pressure were, on the average, insignificant.

5. The subjects who showed a marked increase in threshold during the minor stages of anoxia reacted in a similar way with increasing O_2 deprivation in relation to the other subjects of the group, the correlation by the rank difference method between the subjects who were tested at altitudes I (7,400 ft.) and II (11,000 ft.) being 0.76 ± 0.07, for I and III (15,000 ft.) being 0.71 ± 0.08, and for II and III being 0.88 ± 0.03.

6. For the subjects studied under the conditions of these experiments, thresholds relating to dark adaptation provide a sensitive test for showing the initial effects of anoxia at partial pressures of O_2 where other sensory

tests have failed to show reliable changes. However, these effects are probably of no great practical significance in relation to night blindness in a pilot until altitudes of approximately 10,000 to 12,000 feet are attained.

7. In our opinion, the results obtained in this experiment suggest that the changes are not concerned with the photochemical substances of the retina but with the neural elements of both the retina and the central nervous system.

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RELATION BETWEEN ELECTROLYTE IMBALANCE AND EXCRETION OF AN ANTI-DIURETIC SUBSTANCE IN ADRENALECTOMIZED CATS

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Recent publications (6, 11, 15) have suggested that the adrenal cortex is related to the anti-diuretic function of the posterior lobe of the pituitary. Silvette and Britton believe that the adrenocortical hormone serves as an antagonist to the anti-diuretic action of the posterior pituitary in its influence upon the excretion of the water and sodium chloride. Winter, Ingram and Gross (16) report that injections of pitressin in cats, with diabetes insipidus or with diabetes insipidus and hypoadrenia, play an important part in the survival period and changes in electrolyte level and water balance of these animals. Such evidence suggests that the posterior lobe participates in both water and electrolyte exchange. Inasmuch as such action is also attributed to the adrenal cortex, a relationship of function of the two glands appears probable. The exact nature of such a relationship has not been studied.

A direct approach to the problem is to determine variations in the excretion of an anti-diuretic hormone of the posterior pituitary during hypoadrenia. Such a study has been made possible by Burn (3) who devised a technique for determining the presence of this substance in blood and urine. Using a modified form of this procedure, Gilman and Goodman (7, 8) have shown that the anti-diuretic principle is excreted in the urine during dehydration. This has been recently confirmed (1, 9). Because dehydration also occurs in hypoadrenia, it appeared possible to establish a relationship between the adrenal cortex and posterior pituitary by determining *1*, if an anti-diuretic principle is excreted during suprarenal insufficiency, and *2*, its relationship to electrolyte imbalance. A preliminary report of this investigation has appeared (10).

PROCEDURE. The daily fluid intake and output, serum sodium and potassium and urinary excretion of an anti-diuretic substance was determined in 12 adult cats during each of the following periods: *1*, control interval of 5 to 16 days; *2*, 24 to 72 hours of dehydration; *3*, 5 to 9 days of injections of a maintenance dose of Wilson's cortical extract[1] (0.75–1.5 cc.

[1] We are indebted to Dr. David Klein of the Wilson Laboratories for his generous supply of adrenal cortical extract.

per day) following a two stage adrenalectomy, and 4, 24 to 72 hours after cessation of such injections. In a few animals, observation 4 was repeated upon restoring the animal's condition to control levels of serum sodium with cortin. All cats died 4 to 8 days after cortin administration was stopped. Exact fluid balance was not determined since the water content of the food, feces and expired air was not calculated. Daily bovex[2] rations were kept constant throughout the investigation and except in hydration or dehydration experiments, water was given *ad lib.*

The serum sodium (4) and serum potassium (2) were determined from intracardial samples of blood drawn at the time of each urine collection.

The presence of an anti-diuretic principle in 24 hour samples of urine collected from cats during the periods noted above and from 36 non-dehydrated rats, was assayed by the modified technique of Gilman and Goodman (8). Over 200 bioassays were made in this study using a total of 180 adult male rats. Each urine sample was made acid with 1 per cent acetic acid, dialyzed through a cellophane bag against running tap water for 3 to 5 hours until chloride-free and then concentrated in vacuo to 10 to 16 cc. This volume was then injected subcutaneously in equal amounts to a group of 4 adult male rats previously hydrated by gavage (8 per cent of body weight) with tap water. Control rats used for assay were injected with distilled or tap water. In conformance with previous reports, the measure of diuretic activity of the injected fluids was regarded as the time of excretion in minutes (index of diuresis) of 50 per cent of the total fluid administered for hydration to a group of 4 rats. An increase of 30 minutes or more above this time interval was accepted as a definite indication of anti-diuretic activity.

In another phase of this problem a low serum sodium was produced in 6 cats by the intraperitoneal glucose injection method of Darrow and Yannett (5). The animals were given a constant bovex ration and free access to water. Records were kept of water intake and output and determinations made of serum sodium and of the anti-diuretic principle in urine.

Results. In preliminary studies, it was found that hydrated rats in groups of 4 excreted one-half of the tap water given by stomach tube at an average of 95.4 minutes (78–115 range). This value is in fair agreement though slightly higher than that reported previously (1, 8). When such rats, however, were used for bioassay of an anti-diuretic principle, and were given subcutaneous injections of dialyzed, concentrated urine obtained from 15 normal cats and 36 normal rats, the diuretic time was 102 and 111 minutes respectively. These figures were regarded as controls representing absence of an anti-diuretic principle in the injected urine inasmuch as no appreciable difference in time was noted when distilled or

[2] Bovex was obtained from the Old Trusty Dog Food Company, Needham Heights, Massachusetts.

tap water was substituted for the injected urine. When the rats were dehydrated 24 to 48 hours, the urine samples showed a marked increase in excretion time of 157 and 193 minutes respectively. This finding was interpreted to indicate presence of an anti-diuretic principle in the urine.

The effects of dehydration and adrenalectomy on the fluid exchange, serum sodium and potassium and excretion of an anti-diuretic substance

TABLE 1

Fluid exchange, electrolyte balance and anti-diuretic action of urine from 12 adult cats before and after complete adrenalectomy

PROCEDURE	NUMBER OF OBSERVATIONS	TIME	DAILY FLUID, AVERAGE		BLOOD SERUM		INDEX OF DIURESIS, AVERAGE*	REMARKS
			Intake	Output	Na	K		
Before adrenalectomy								
		hours	*cc.*	*cc.*	*mgm. per cent*	*mgm. per cent*	*min.*	
Water ad lib	96	24	123	86	331.0 (321-349)	16.5 (10.5-21.0)	106.6 (86-120)	Only 27 tests made on serum Na, K and a-d principle
Dehydration	21	24	0	45	326.8 (318-342)	15.1 (11-18.5)	160.6 (125-185)	Constant bovex ration all eaten
		48	0	22	322.7 (318-341)	15.1 (9.5-20)	184.4 (150-220)	
		72	0	22	327.9 (318-334)	17.3 (11-23)	242.5 (189-450)	
After adenalectomy								
Water ad lib	12	24	110	67	326.0 (319-348)	16.9 (13-20)	109.8 (91-119)	Maintenance cortin given
	23	24	68	30	321.3 (305-333)	16.7 (12-20)	142.5 (124-180)	Maintenance cortin stopped
		48	41	33	307.6 (270-334)	18.8 (14-23)	204.5 (133-295)	
		72	29	23	299.7 (258-308)	19.5 (12-30)	258 (179-381)	
Forced fluid by stomach tube	8	24	100	68	326.5 (318-339)	14.8 (10-19)	129.4 (111-143)	All food eaten; maintenance cortin stopped
		48	100	48	305.0 (273-320)	17.6 (13-21)	174.5 (150-206)	Occasional vomiting; maintenance cortin stopped
		72	100	27	282.6 (247-301)	24.8 (16-32)	239.0 (203-315)	Occasional diarrhea and vomiting; maintenance cortin stopped

* Number of minutes required for group of 4 hydrated rats to excrete one-half the amount of fluid given by stomach tube after subcutaneous injection of a dialyzed concentrated urine of cats.

in 12 adult cats are summarized in table 1. During the control period in unoperated cats, there was a normal fluid exchange and electrolyte balance. Daily urine samples showed an average normal *index of diuresis* of 106.6 minutes and, therefore, contained no anti-diuretic principle. Following dehydration of 24 to 72 consecutive hours, the daily decreased urinary output was not accompanied by any significant change in serum sodium or potassium. Some of the values at the 72 hour period were

slightly above that of the control but still within the normal range. All cats ate their daily ration throughout the dehydration period. Daily urine samples, however, contained an anti-diuretic principle which was present in progressively greater amounts as dehydration continued. This is indicated by the increased average *diuretic time* of 160, 184 and 243 minutes for the different periods of dehydration respectively. Such time values correspond to the action of 88, 107 and 248 milli-units of pitressin as determined by the injection of such amounts into a group of hydrated rats. At the end of this experiment these cats were given fluid *ad lib* and assays on urine samples revealed an absence of an anti-diuretic substance.

Table 1 also demonstrates that adrenalectomized cats maintained on adequate doses of cortin for 5 to 9 days post-operatively showed approximately normal intake and output and serum sodium and potassium and absence of an anti-diuretic substance in the urine. Following cessation of maintenance doses of cortin, significant changes in these observations were noted. The water intake and output gradually diminished and as previously noted (14) there was a marked decrease in water intake and output with a tendency toward a negative water balance. The electrolyte imbalance was typical of hypoadrenia except that the rise in serum potassium was slight at 72 hours. Daily urine samples definitely contained an anti-diuretic substance as indicated by the prolonged index of diuresis (142, 204 and 258 min.). The appearance of this substance within 24 hours after cortin was discontinued seemed to be associated chiefly with decreased water intake inasmuch as the food intake and electrolyte blood level at this time were similar to those in the control period. There was only one exception, noted in a cat whose serum sodium was 305 mgm. per cent at the end of the first 24 hours. Urine samples taken at 6 and 12 hours after cessation of cortin did not contain any anti-diuretic principle. At 48 and 72 hour intervals, the diuretic index of the urine samples was markedly increased more so than after simple dehydration noted above (table 1). It is significant that at these intervals the cats still ingested some water and showed a variable but moderate decrease in appetite for their bovex ration. The increase in the index of diuresis appears now to be due not only to decrease in water intake but also to the gradual decrease in serum sodium.

To evaluate the relative importance of water intake and electrolyte imbalance in stimulating the excretion of an anti-diuretic substance, fluids were forced by stomach tube to 6 adrenalectomized cats after cessation of maintenance cortin injections. Table 1 shows that such a procedure markedly decreases the excretion of an anti-diuretic principle during the first 48 hours even though the serum sodium was below normal in most cases on the second day. At the end of the 3rd day, the effect of giving fluids by gavage was absent due probably to decreased absorption

of water from the gastro-intestinal tract as well as to the lower blood sodium.

Decreased serum sodium in conditions other than hypoadrenia may stimulate the urinary excretion of an anti-diuretic substance. Table 2 summarizes such results in 6 adult cats whose serum sodium was reduced by intraperitoneal injections of isotonic glucose and the removal of an equal volume of fluid by abdominal paracentesis. The extent of sodium depletion was variable with resulting corresponding variations in fluid exchange and excretion of an anti-diuretic substance. However, in each individual case, the general trend corresponded with that indicated by the average of figures in table 2. These figures show that the more marked the sodium depletion, the greater the excretion of an anti-diuretic substance. During the first 24 hours of low serum sodium, there was a greater urinary

TABLE 2

Effect of sodium depletion on the excretion of an anti-diuretic substance in urine

PROCEDURE	TIME	DAILY FLUID, AVERAGE		BLOOD SERUM SODIUM	INDEX OF DIURESIS, AVERAGE
		Intake	Output		
	hours	*cc.*	*cc.*	*mgm. per cent*	*min.*
Control	24	137	93	329.9 (322-332)	109.5 (99–119)
Sodium depletion	24	51	61	290.5 (238–302)	299 (181–500)
	48	79	36.5	303.6 (255–322)	188.6 (159–315)
	72	113	27	315.8 (302–329)	147.5 (122–195)

excretion of the substance during the same interval after dehydration of normal cats or after cessation of maintenance doses of cortin in adrenalectomized cats (see table 1). This is attributed to the greater sodium loss with a more marked resulting negative water balance. The animals during the first day drank little and ate sparingly. One cat, showing serum sodium values of 238 and 255 mgm. per cent at 24 and 48 hours respectively, died on the 3rd day of the experimental period.

During the 2nd and 3rd days a gradual rise of serum sodium was noted. This electrolyte shift was due to a positive water balance and a more normal appetite for bovex rations. The excretion of an anti-diuretic substance in the urine fell markedly although it was still present at the end of the 3rd day.

Discussion. It is well known that among other actions, the posterior pituitary secretes an anti-diuretic principle. The need for water conservation by the body appears to be a potent stimulus for this function and

an excess of this principle may be found in urine during dehydration (1, 8, 9). Contradictory results have been reported (13). Because such a substance is absent in dehydrated animals following hypophysectomy, Gillman and Goodman believe it is of pituitary origin. Our data confirm the finding of such a principle in the urine of dehydrated cats and, in addition, show that the appearance of this substance during the first 72 hours of dehydration is not related to serum electrolyte imbalance.

Both the posterior pituitary and adrenal cortex are known to be concerned with water metabolism of the body. Since cats tend to show a negative water balance after complete adrenalectomy (14), it is not surprising to find that they should excrete an anti-diuretic substance in the urine. Dehydration, therefore, occasioned by suprarenal insufficiency, stimulates posterior pituitary activity. That this is of primary nature is shown by the fact that such stimulation may be present during the early period of hypoadrenia when no electrolyte imbalance is noted; secondly, it is significantly diminished upon increasing fluid intake by gavage in adrenalectomized cats.

The influence of electrolyte changes on water balance is fully appreciated and is reflected in our results in the more marked increased excretion of the anti-diuretic substance in adrenalectomized cats when the serum sodium fell as compared to the excretion in unoperated dehydrated cats. This influence is secondary to the dehydration caused by simple fluid imbalance. That low serum sodium can increase the degree of dehydration already present (5) and thus augment stimulation for excretion of an anti-diuretic substance has also been shown by our experiments.

Dehydration in our adrenalectomized cats appears to have been self-imposed inasmuch as water was always available. The mechanism of this phenomenon during the first 24 hours of hypoadrenia and when electrolyte imbalance is absent, is explained in part by a decrease in fluid intake to about two-thirds of their normal amount. However, the fact that there was a proportionally greater decrease in urinary output during this period indicates that significant amounts of fluid were not excreted. The dehydration, therefore, is probably also caused by a shift of vascular fluid to extracellular and then to intracellular spaces due to early changes in capillary permeability. Such a concept of adreno-cortical function has been repeatedly emphasized (12).

The relationship between the posterior pituitary and the adrenal cortex may be considered direct only insofar as they both appear to be concerned with water balance of the body. Dehydration appears to be the common factor in the activity of both glands. Further, both glands are known to influence capillary actions. The absence of the cortical hormone in hypoadrenia may in part be compensated for by over-production of an anti-diuretic principle for the conservation of body water. This may continue until other complicating factors such as electrolyte imbalance

with renal loss of sodium, vomiting, and diarrhea augment the dehydration and produce a vicious cycle in which no amount of anti-diuretic substance secreted can maintain normal water balance of the body.

SUMMARY

1. Dehydration of cats and rats results in the urinary excretion of an anti-diuretic substance, confirming previous results.

2. No significant variations in serum sodium and potassium are found accompanying this excretion during the first 24 to 72 hours of dehydration in cats.

3. Adrenalectomized cats maintained on adequate doses of cortin do not excrete an anti-diuretic substance. However, such a substance is found in the urine 24 hours after cessation of cortin therapy. Its appearance at this time is largely due to decreased fluid intake, and not to changes in serum sodium or potassium. Forty-eight and 72 hours after cortin is discontinued and after a definite electrolyte imbalance is noted, much larger quantities of the anti-diuretic substance are excreted.

4. Decreased serum sodium in conditions other than hypoadrenia similarly results in excretion of large amounts of the anti-diuretic substance.

5. It is believed that the functions of the adrenal cortex and the posterior pituitary are related directly insofar as they are both concerned with water metabolism. Following adrenalectomy, there is an initial stimulation of the posterior pituitary due to early dehydration and a secondary augmentation caused by electrolyte imbalance.

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LEUKOCYTOSIS FOLLOWING PARENTERAL ADMINISTRATION OF LIVER EXTRACT IN MAN

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Murphy (1932) reported that liver extract administered intravenously or intramuscularly to pernicious anemia patients produced within eight hours a transient increase in leukocytes which was distinct from the later slow recovery of leukocytes to normal levels coincident with remission. Subsequent investigations of this prompt, transient leukocytosis in the normal human (Powers, Murphy and Humphreys, 1933; Meyer, Middleton and Thewlis, 1934; Powers, 1935) have demonstrated that the leukocytosis was the result of both an absolute and relative neutrophilic increase, and was independent of moderate body activity and diurnal variations, since the peak of the rise regularly occurred six to eight hours after the time of injection in both ambulatory and bed subjects. The height of the rise in individuals varied from 20 per cent to 185 per cent. With intravenous administration the leukocytosis was more prompt (3 hours to the peak) but less well sustained; the typical rise occurred in the absence of the spleen and in chronic and acute neutropoenia.

The neutrophilic increase has not been proven the result of an increase in number of young cells of the series (Meyer, Middleton and Thewlis, 1934; Powers, 1935).

Dedichen (1936, 1936a) reported that the height of the leukocyte rise was dependent on the amount of liver extract injected and that young people responded better than older people. He reported that Laland and Klem's extract of liver, which is active in pernicious anemia in 0.2 mgm. dry weight quantities, was also capable of giving rise to the leukocytosis, whereas inactive fractions did not occasion the leukocytosis.

Dedichen (1936a) proposed the use of swine for the laboratory assay of liver extract based on the leukocyte increasing effect, after having discarded rabbits, dogs and a sheep as unsatisfactory. It must be noted, however, that swine normally have a leukocyte count of between 15,000 and 25,000 and that the minimal amount of fresh liver (of which the extract is derived) to produce a leukocytosis in pigs is estimated to be more than three times that required for the human. He obtained "uneven results." No mention of differential counts was made in relation to the above work.

The leukocyte-increasing effect of liver extract, therefore, suggests a possible means of bioassay of the antipernicious anemia principle in present day commercial extracts provided that the reaction can be demonstrated to be specific for the principle.

METHOD. Blood for counting and smears was obtained from free flowing puncture wounds of finger tips used in succession; re-punctures of finger tips were as widely separated as possible. Two Trenner automatic pipettes[1] were filled with blood and diluted with Turk's fluid. Double chamber counts were made on each pipette; the recorded final value was the average count of sixteen 1 mm. sq. areas. Multiple counts on single specimens of blood, using ten pipettes and making double counts, consistently showed coefficients of variation between 5 per cent and 9 per cent. The smears were made by the slide technique and stained with Wright's stain. Complete differential counts were made on 200 leukocytes and recorded as per cent values from which the absolute number was calculated. All counts were made by one observer.

Four control counts spaced at two and three hour intervals were made on the day preceding injection, and similarly spaced counts were made on the day after injection. On the day of injection, counts were made at hourly intervals from 8 a.m. through 8 p.m. The injections were made immediately after the second count at 9 a.m. There were available, therefore, six counts for control purposes.

Each of four normal males 22 to 27 years old received the six injections below with at least one month interval between injections. The sequence of injection was not selected. During the three-day period only random activity was permitted and drugs other than nicotine and caffein were avoided. The subjects were clinically in good health and showed no abnormal temperature variations at any time.

The following sterile preparations made to 3 cc. volume were injected deep intragluteally using alternate hips: 1. A commercial liver extract, formerly labeled 3 cc. derived from 100 grams of liver, now 10 units per 3 cc. (this extract was used by the first three groups of investigators previously noted). 2. A commercial liver extract formerly labeled 1 cc. derived from 100 grams of liver, now 15 units per 1 cc. 3. A kidney extract prepared by the commercial methods used in making liver extract no. 1 above, which was known to be inactive by trials on human pernicious anemia. 4. Forty milligrams "Anahemin" obtained from British Drug Houses; it is the Reinecke salt and $(NH_4)_2SO_4$ precipitate fraction as prepared by Dakin and West (Dakin and West, 1935; Ungley, Davidson, and Wayne, 1936). 5. Twenty milligrams fraction H (Subbarow, Jacobson and Prochownick, 1936; Jacobson and Subbarow, 1937). 6. Physi-

[1] Pipettes and hemacytometer were certified by the U. S. Bureau of Standards.

ologic saline with 0.5 per cent phenol. Each 3 cc. volume was estimated to contain approximately equivalent anti-anemic potency, if any.

RESULTS. The six control counts preceding each injection were averaged to serve as a basis of comparison of the effects of that injection. The results of the separate injections into each subject are presented in table 1 showing the peak increase in total number of leukocytes expressed in per cent above the average control level and the number of hours elapsed after injection at which the peak occurred; the figures in parentheses indicate the number of hours elapsed until a value within the range of error of the peak value was reached. The averages of the peak values obtained with each extract indicate that the least purified liver extract and the inactive kidney extract had the most marked effect on the number of leukocytes; they were followed by the "Anahemin," the fraction H, and the 1 cc.

TABLE 1

The per cent increase above control-average of peak value of total leukocytes and elapsed time in hours for occurrence of peak value in response to injections

SUBJECT	LIVER EXT., 3 CC.		LIVER EXT., 1 CC.		FRACTION H, 20 MGM.		"ANAHEMIN" 40 MGM.		KIDNEY EXT., 3 CC.		SALT SOL., 3 CC.	
	P.I.*	E.T.†	P.I.*	E.T.†	P.I.*	E.T.†	P.I.*	E.T.†	P.I.*	E.T.†	P.I.*	E.T.†
Be	105	10(8)	15	11(2)	56	5(−)	50	10(4)	78	10(8)	0	(−)
Br	61	11(7)	63	9(8)	62	7(−)	74	4(3)	89	7(6)	35	11(9)
Ca	94	7(6)	34	5(−)	71	8(−)	38	7(5)	103	5(−)	26	2, 6, 7
Ki	88	5(−)	75	10(5)	53	6(−)	67	5(−)	79	4(−)	28	4(−)
Average	87	8.3	47	8.8	60	6.5	57	6.5	87	6.5	22	6.3
Composite	70	8(5)	30	8(6)	38	9(5)	50	5(4)	77	7(5)	20	9(7)

* Per cent increase in peak value above control average.

† Elapsed time for occurrence of peak value; the numbers in parentheses refer to time elapsed for attainment of a value within the range of error of peak value.

liver extract. There was also some rise in the number of leukocytes following the injection of salt solution.

A composite curve for each extract was constructed by averaging similarly timed values. The same sequence of activity resulted, although the peak values were not as high due to differences in rates of ascent of the separate curves and the tendency to cancellation-smoothing. A distinct leukopoenic phase immediately after the injection was observed in but approximately ⅓ the instances and therefore is probably a chance variation. The peak of the rise was generally obtained within 4 to 10 hours after injection and usually about the sixth hour.

No correlation was observable between the soreness of the injected area and the height or time of maximal response. As others have found, the count usually does not return to entirely normal levels within 31 hours

after injection, but a few counts made on succeeding days indicate that the normal level is reached by the fiftieth to sixtieth hour.

The differential counts of the stained smears agree with the results of previous investigations in that the increased total count was due to both a relative and absolute increase in number of the neutrophil cell series. The other series of leukocytes showed no consistent changes or irregular variations in the absolute counts. The cells of the neutrophil series were arbitrarily separated into mature and immature forms by combining all the neutrophil cells younger than the bi-lobed cells into the immature group and the older segmented or lobed cells into the mature group. Assuming ideally that no error or variation is introduced in collecting the sample, making the smear and from irregular distribution of cells on the smear, and that absolute identification is possible, there remains the chance error of random sampling (Barnett, 1933; Goldner and Mann, 1938). Comparison of the highest immature relative count of the six control counts with the highest immature relative count after injection by the standard deviation of a proportion method showed that the difference could reasonably occur by chance due to sampling in all but 5 of the 24 tests; in 3 of the 5 instances the significant difference appeared *after* the peak total rise.

Discussion. The above data demonstrate that the increase in leukocytes following the intramuscular injection of liver extract is not a specific effect of the anti-pernicious anemia principle. Further, the height of response to approximately equivalent potency tends to decrease with increasing purification. Stockinger and Berchtold (1937) have observed almost identical increases in the leukocyte count following the injection of Prahormon, an anterior pituitary preparation. Krusen (1937) observed similar prompt leukocyte rises without change of the filament-nonfilament count after fever produced by diathermy. Dedichen (1936a) has observed "a full normal rise in the leukocyte count following an injection of 5 cc. of milk." Hunt et al. (1935) have reported similarly timed leukocytoses following the injection of typhoid antigen.

Since the data do not give evidence of active granulopoiesis, the increase in leukocytes can be regarded as a redistribution phenomenon, dependent on unidentified substances contained in the preparations. Timed as the leukocytosis is, there is apparent no reliable explanation of the mechanism of a redistribution under the above circumstances although the concept of distribution leukocytosis seems well established (Garrey and Bryan, 1935). The rapid rhythms and turnover of the white cell picture in normal persons as described by Sabin et al. (1925) suggests, however, a possible means whereby granulopoiesis might be stimulated by the extracts but without the methods used giving evidence of the process. Moreover, an increased rate of maturation would not be detectable. Silverman (1938) has found that a chemical substance derived from skin is capable of in-

ducing positive chemotaxis of leukocytes in in vitro experiments, and like the materials in liver extract is water soluble and readily adsorbable on kaolin. It is possible that some such material may be present in the extracts, but would be highly diluted by the blood stream. Doan (1932) considers that the leukopoietic effect of nucleic acid and its derivatives is exerted by virtue of a chemotactic action on the older myelocytes. Focal reactions are eliminated from consideration, since intravenous administration acts similarly to intramuscular injection.

Murphy (1932) has observed the prompt response to occur in pernicious anemia, in which condition (as also in chronic neutropoenia) there is a marked deficiency, but not complete absence, of leukocyte maturation; true restoration of the peripheral leukocyte counts in such patients is observed to begin 10 to 14 days after the institution of liver therapy. In true agranulocytosis (Foran et al., 1933; Tihen, 1936; Das Gupta and Witts, 1937) on the other hand, continued massive doses of liver extract must be administered for at least 48 to 60 hours before granulopoiesis becomes evident. The comparison of these two pathological states suggests that where the granulocytes are not entirely absent, they may be mobilized, although not stimulated to increase production. Murphy's data show, also, that with spaced repeated injections into pernicious anemia patients the height of response decreases; Dedichen has observed this with repeated injections into a sheep.

The oral administration of large quantities of liver and liver preparations to normal humans for as long as 30 days has been found to cause no significant changes in the leukocyte count (Cornell, 1928; Watkins et al., 1928; Neidhardt and Bannasch, 1929).

The salt-phenol solution control experiments indicate that a portion of the rise might be attributed to a diurnal rhythm, but in agreement with Powers, Murphy and Humphreys probably is not conditioned by such a rhythm. Inasmuch as the granulopoietic system may possess varying degrees of lability, a valid objection might be raised to the semi-quantitative comparisons made above.

Wilson and Carey (1937) have reported that repeated intramuscular injections of liver extract were of value in reducing the mortality rate of pneumonia patients having low or falling leukocyte counts, and, as noted above, injections of liver extract are effective in inducing remission of agranulocytic angina. The lack of evidence of true bone marrow stimulation during the prompt response does not invalidate the clinical therapeutic effectiveness of liver extract in more slowly raising the leukocyte count.

SUMMARY

1. The increase in leukocytes per cubic millimeter in peripheral blood occurring within 10 hours after the intramuscular injection of a commer-

cial liver extract was investigated as a possible means of bioassay of the antipernicious anemia principle.

2. The leukocytosis has been shown to be non-specific in that it occurred following the injection of a kidney extract known to be inactive in pernicious anemia. The increase tended to be less marked with more purified active liver extracts.

3. Evidence of increased granulocyte production preceding or at the peak of leukocytosis was not obtained.

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EFFECT OF PITRESSIN INJECTIONS UPON THE SERUM ELECTROLYTES AND WATER EXCHANGE OF CATS WITH DIABETES INSIPIDUS AND ADRENAL INSUFFICIENCY

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In previous papers (1, 2) we have shown that the serum electrolytes in cats with diabetes insipidus respond differently to adrenal insufficiency than do those of non-polyuric animals, that adrenal removal reduces the fluid exchange in diabetes insipidus, and that cats with diabetes insipidus survive adrenalectomy a shorter time than do non-polyuric cats. It was suggested that such results indicated a possibility of a reciprocal relationship between the posterior lobe of the pituitary and the adrenal cortex, so far as salt and water regulation in the organism is concerned. Silvette and Britton (3) regard these two glands as antagonistic in their action on salt and water excretion. Gersh and Grollman (4), on the other hand, argue against the possibility of a relationship between these organs because they failed to find any histological change in the posterior lobe following adrenalectomy in animals otherwise normal. It might be pointed out, however, that the failure of loss of one organ to affect the histology of the other does not preclude the possibility that their functions may be complementary so far as salt and water regulation is concerned. Furthermore, it is not necessary to imply that the surviving member of such a pair need show hyperfunction. Normal function of such a surviving gland, without the balance usually exerted by the other, may lead to excessive loss of electrolytes. That a hyperfunctioning of the posterior lobe may follow adrenalectomy, however, has been indicated by the results of Martin et al. (5); proof of this is not yet complete.

Our present series of experiments deal with the effects of pitressin[1] injections upon the serum electrolytes and water exchange in normal and in diabetes insipidus cats before and after adrenalectomy. Many investigators (6–13) have studied the effect of posterior lobe injections upon serum electrolytes, with varying results, using large doses of extract and comparatively short periods of observation. In our experiments, we have

[1] The authors are grateful to Dr. Oliver Kamm of Parke, Davis and Company, Detroit, Michigan, for generous supplies of Pitressin used in this study.

used small doses at frequently repeated intervals, in an attempt to simulate physiological conditions as nearly as possible. Even so, the dosages used undoubtedly produced concentrations of hormone exceeding those usually present in the body. Pitressin was injected subcutaneously, three pressor units three times daily. Such dosage was found to be adequate in controlling the polyuria of diabetes insipidus, the urine volume approaching or attaining the normal level after the second day. There was little effect on the water exchange of normal cats. Control blood samples for analysis were obtained in non-adrenalectomized cats before pitressin injections, and another sample three to eight days after beginning pitressin therapy. In the animals used for adrenalectomy, the pitressin was continued during the period of insufficiency, the final blood sample being taken when the animal appeared to be near death.

The cats were fed a uniform diet of fresh ground beef and milk. Diabetes insipidus was produced by lesions in the hypothalamus, using the Horsley-Clarke stereotaxic instrument according to the technique of Fisher, Ingram and Ranson (14). The lesions produce degeneration of the supraoptico-hypophyseal tract with resulting denervation and atrophy of the pars nervosa of the pituitary. Adrenals were removed in two stages by a dorsal approach. Blood for analysis was obtained from the unanesthetized animal by cardiac puncture. The methods used for analysis of the serum electrolytes have been referred to elsewhere (1). In the experiments on water exchange, daily check was made of food and water intake, including the water content of the meat and milk and the calculated metabolic water. Urine volume was measured daily, but fecal water and insensible water loss were not determined, as they were considered to be fairly uniform under the conditions of the experiments.

Observations. The data on serum electrolytes, as shown in table 1, may be summarized as follows:

Cats with diabetes insipidus have normal concentrations of serum sodium, potassium, and chloride. This was found to be true of animals from which blood samples were taken shortly after the appearance of polyuria, as well as for those in which the polyuria had existed for two months or more.

Pitressin injections, in the dosage which we used, did not affect the concentration of serum electrolytes before adrenalectomy either in non-polyuric or in diabetes insipidus animals. The polyuric cats received an amount of pitressin which was adequate to control the polyuria, and the non-polyuric animals received the same amount added to a presumably already adequate supply from their own posterior lobes. Therefore, it may be said that our series of cats embrace a range of hormone content in the animal's body from a grossly inadequate supply to one considerably above normal (unless the injection of pitressin in the normal animal in-

hibited the production of hormone from his own posterior lobe), and yet in the whole range the serum electrolytes remain remarkably constant.

Following adrenalectomy, there is a significant decrease in the concentration of serum sodium and chloride in the non-polyuric animals, but not in the diabetes insipidus cats; this confirms our previous report (1). Pitressin therapy in the polyuric cats after adrenal removal leads in most of the animals to a fall in serum sodium and chloride comparable to that which occurs in ordinary adrenalectomized animals. There is considerable individual variation; the individual data show that there was one cat in each of the four groups following adrenalectomy which failed to conform to the electrolyte pattern of its particular group. This is, perhaps, to be expected, since in the diabetes insipidus cats there were no doubt varying degrees of posterior lobe insufficiency; also, all the cats

TABLE 1

Median values for serum electrolytes of cats, in milliequivalents per liter

Numbers in parentheses indicate number of animals used

KIND OF CATS	SERUM ELECTROLYTE	WITHOUT PITRESSIN			WITH PITRESSIN		
		Before adrenalectomy	After adrenalectomy	Per cent change	Before adrenalectomy	After adrenalectomy	Per cent change
Nonpolyuric controls	Sodium	152.2 (21)	125.0 (4)	−17.9	151.8 (4)	131.4 (4)	−13.5
	Chloride	115.6 (21)	105.7 (4)	−8.6	115.8 (4)	102.0 (5)	−11.8
	Potassium	6.4 (21)	9.4 (4)	+46.9	6.9 (4)	9.7 (3)	+40.6
Diabetes insipidus	Sodium	148.3 (17)	142.3 (8)	−4.0	147.9 (5)	128.9 (7)	−12.9
	Chloride	117.0 (18)	116.4 (8)	−0.5	114.4 (5)	98.4 (7)	−13.9
	Potassium	6.0 (16)	9.3 (6)	+45.0	6.2 (5)	10.7 (6)	+72.6

receiving pitressin were given identical doses, although it is not improbable that their pitressin requirements were not in all cases the same. Increase in serum potassium following adrenalectomy was found in all cats without exception. The characteristic increases in blood urea and blood concentration were found in all the groups of cats following adrenal removal.

It has already been pointed out (1, 2) that the survival time of polyuric cats following adrenalectomy is much shorter than that of non-polyuric animals. Our present work confirms this (table 2), and further shows that pitressin does not, on the average, markedly affect the survival time of non-polyuric animals. It does, however, prolong that of the diabetes insipidus cats to about the same as the non-polyuric. Since the polyuric cats without pitressin are the only ones that show no change, on the average, in serum sodium and chloride following adrenalectomy, and also have a much shorter survival time than the others, it might be argued that the fall in serum sodium and chloride is a function of the time elapsed since

adrenal removal, and the polyuric cats do not show this change simply because they don't live long enough. We have already partially answered such a possible argument (1), and we now have additional evidence that the survival time is not important in serum electrolyte changes following adrenal removal. A careful check of our individual data in an attempt to correlate terminal serum sodium and chloride concentration with survival time in each of the four groups of adrenalectomized animals fails to show that such correlation exists. For example: five of the diabetes insipidus cats without pitressin survived adrenalectomy from three to five days; there were also five animals in all the other groups combined which survived an equally short length of time; the terminal serum chlorides in the former showed a median value of 116 milliequivalents per

TABLE 2

Water exchange of cats

KIND OF CATS	CONDITION	NUMBER OF ANIMALS	DAILY AVERAGE			AVERAGE DAILY "COMPARATIVE WATER BALANCE"	AVERAGE SURVIVAL	AVERAGE TOTAL "COMPARATIVE WATER BALANCE"
			Water intake	Urine output	"Water retention"			
			cc.	*cc.*	*cc.*		*days*	
Diabetes insipidus	Before adrenalectomy	19	515	442	73			
	After adrenalectomy	10	119	140	−21	−94	2.9	−260
	After adrenalectomy with pitressin	9	153	132	21	−52	6.8	−331
Controls	Before adrenalectomy	10	172	125	47			
	After adrenalectomy	4	73	61	12	−35	8.3	−289
	After adrenalectomy with pitressin	6	96	98	−2	−49	6.7	−309

liter and in the latter 92; the corresponding figures for sodium are 142 and 128 respectively.

It is evident, then, that the survival time of the polyuric cats in adrenal insufficiency is adequate for the occurrence of a fall in serum sodium and chloride. The fact that such a fall does not take place in such animals while it does in similar animals treated with pitressin suggests that the posterior lobe of the pituitary plays a specific rôle in the reduction of serum electrolytes in adrenal insufficiency, and that the posterior lobe secretion responsible for such action is present in extracts containing the pressor principle, and may be identical with the latter. These results also suggest that the posterior lobe may be concerned with electrolyte excretion in the absence of the adrenals and experiments to test this are now in progress.

Of the three groups showing significant decreases in serum sodium and chloride after adrenal removal, the two groups receiving pitressin suffered a nearly equivalent loss of the two electrolytes, while the untreated non-polyuric adrenalectomized cats lost about twice as much sodium as they did chloride. The latter result is in agreement with Baumann and Kurland (15) and Harrop et al. (16). Further work is indicated before attempting an explanation of these results.

Table 2 summarizes the results of the experiments on water balance. "Water retention" is the water intake minus the urine volume; "comparative water balance" is the water retention after adrenalectomy minus the water retention before adrenalectomy. "Total comparative water balance" is the water retention for the entire post-adrenalectomy period compared with the pre-adrenalectomy retention for the same number of days in the same animals.

The post-adrenalectomy water retention is in all cases lower than the pre-adrenalectomy. Pitressin administered after adrenalectomy to the non-polyuric cats did not markedly affect the post-adrenalectomy water balance or the survival time; the differences that appear in the table between the non-polyuric cats with and without pitressin after adrenalectomy are within the range of individual variation.

Untreated polyuric cats have a more strongly negative post-adrenalectomy daily water balance than any of the other groups, and a much shorter survival time; pitressin therapy brings both within the range of the adrenalectomized control cats. The water loss, however, does not appear to be the cause of the early death, because the total water loss before death is less in the untreated polyuric cats after adrenalectomy than in any of the other groups. Therefore, the action of pitressin in lengthening the post-adrenalectomy survival time of cats with diabetes insipidus cannot be due entirely to its effect on the total water balance of the organism.

Normal cats when almost completely deprived of fluid may survive for long periods of time; they can apparently maintain a fairly normal circulatory medium by shifts of fluid into the blood stream from extravascular sources. In the absence of the adrenals a negative water balance is set up, but this in itself is not sufficient to cause death, since comparable loss may be suffered by normal animals with safety (2). If, however, the negative balance is accompanied by inability to shift fluid into the blood, or if the shift occurs even in the opposite direction, as maintained by several investigators (17–20), circulatory failure may result due to concentration of the blood.

Cats with diabetes insipidus when deprived of fluid suffer rather rapid depletion of body fluid because they continue to excrete a marked excess of water in the urine. When water can no longer be mobilized from extra-

vascular sources they die with physical symptoms resembling adrenal insufficiency, presumably due to concentration of the blood and failure of circulation. After adrenalectomy, cats with diabetes insipidus have a much more unfavorable daily water balance than do ordinary adrenalectomized cats; although the total loss through the urine is not in itself sufficient to cause death, such loss in combination with a failure of blood dilution from extravascular sources may well cause death to occur more quickly than in ordinary adrenalectomized animals.

The results cited above appear to offer definite evidence of a participation of the normally innervated posterior pituitary in certain phases of the metabolism of sodium and chloride. In the absence of adrenal cortical hormone the posterior lobe hormones may cause a fall in the level of these substances in the blood serum, in spite of a concentration of the blood. When both factors are missing, the serum sodium and chloride remain at normal levels. One must not overlook the fact, however, that some actual loss of electrolytes from the body may take place, since the normal serum levels may be due in part to concentration of the blood; this loss must be much less than occurs normally. However, in spite of the normal serum levels of these substances, death occurs after a relatively short interval. It is difficult to relate the shortness of the survival time or the actual cause of death directly to changes in mineral metabolism. Alterations in water metabolism, with which the former may of course be associated, seem to offer a more logical reason for the circulatory failure which takes place. As has been stated (2), there seems to be a failure of thirst in polyuric cats after adrenalectomy which may be associated with shifts of body fluids; rapid loss of water by way of the kidneys plus shifts within the body produce rapid dehydration of the blood. In other words, the normally innervated posterior pituitary prolongs survival after adrenalectomy by slowing the rate of dehydration of the blood. If this interpretation is correct, then maintenance of a dilute circulatory medium in the absence of both adrenals and posterior lobe should prolong life; experiments along this line are in progress.

SUMMARY

1. Cats with diabetes insipidus have normal concentrations of serum sodium, potassium, and chloride.

2. Pitressin injection, in dosage adequate to control the polyuria of diabetes insipidus, does not affect the concentrations of sodium, potassium or chloride in the serum of normal or of diabetes insipidus cats.

3. In adrenal insufficiency, untreated polyuric cats have a very short survival time, and do not show the usual decrease in serum sodium and chloride, but if pitressin is administered, diabetes insipidus cats have a survival time about the same as ordinary adrenalectomized animals, and

show the usual changes in serum sodium and chloride. The post-adrenalectomy fall in these electrolytes in pitressin-treated cats with diabetes insipidus is not entirely due to the prolonged survival time.

4. Ordinary adrenalectomized cats lose more sodium than chloride from the serum during the insufficiency period; cats treated with pitressin after adrenalectomy appear to lose sodium and chloride in approximately equivalent amounts. Pitressin administration to ordinary adrenalectomized cats does not affect the survival time.

5. All the cats showed negative daily water balance following adrenal removal which was of about the same order of magnitude for non-polyuric cats with and without pitressin, and for diabetes insipidus cats on pitressin, but which was much greater in the untreated polyuric cats. The total water lost during the entire post-adrenalectomy period, however, was slightly less in the latter group. In no case was the total water loss great enough in itself to account for the death of the animal. Abnormalities in internal fluid shifts are probably more important in causing death than changes in the water balance of the whole organism.

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THE BLOOD HISTAMINE DURING ANAPHYLACTIC SHOCK IN THE HORSE AND CALF[1]

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Dragstedt and Gebauer-Fuelnegg (1932) demonstrated the presence of histamine activity in the blood of the supradiaphragmatic inferior vena cava of dogs in anaphylactic shock. Recently, one of us quantitatively determined the changes in the histamine content of the blood during anaphylaxis in the dog and the guinea pig (Code, 1938). A marked increase in the blood histamine occurred during severe reactions and the results indicated that histamine plays a significant rôle in the production of anaphylactic symptoms. These positive findings raised the question of whether or not the liberation of histamine is a fundamental accompaniment of anaphylaxis.

In an effort to clarify this question we have attempted to extend the observations to other species. The larger domestic animals were chosen for this purpose because they could be expected to yield sufficient blood for detailed analysis. In order that the results might be roughly comparable anaphylactic states were induced by procedures similar to those used in the studies on the dog and guinea pig.

METHODS AND PROCEDURES. Animals were sensitized to egg white, horse serum or dog serum by five to fourteen daily intravenous, subcutaneous or alternating intravenous and subcutaneous injections. The egg white solution was prepared by diluting one part fresh egg white with four parts physiologic saline solution and mixing until the gelatinous character of the egg white had disappeared. Samples of blood were taken as a routine from the jugular vein. A rough estimate of the clotting time of the blood was made by noting the time required for coagulation in a clean glass test tube. When an anticoagulant was needed sterile heparin solution (Connaught Laboratories) was used. The histamine concentration of the blood was determined by a modification of the Barsoum-Gaddum method (Code, 1937a) and the results were expressed in terms of

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histamine base. When the distribution of the histamine between the plasma and the cellular constituents of the blood was determined the methods used were those reported in an earlier publication (Code, 1937b).

Essentially the same procedure was followed in all experiments. The effect of the antigen on the unsensitized animals was first tested. The dose of the antigen which was later to be used to evoke shock was given intravenously and the animals were observed for signs of reaction. Samples of blood for estimation of histamine and clotting time were taken before and after the injection. Following this control test the sensitizing injections were given. After an interval of two or more weeks the effects of intravenous injection of the shock dose of the antigen were again observed and the determinations on the blood repeated. When reactions

TABLE 1

The blood histamine of normal and sensitized horses and calves before and after the injection of egg white, dog serum or horse serum

ANIMAL	ANTIGEN INJECTED	γ HISTAMINE PER CC. BLOOD			
		Before sensitization		After sensitization	
		Before antigen injected	10 minutes after antigen injected	Before antigen injected	10 minutes after antigen injected
1. Horse	100 cc. dog serum	0.033	0.029	0.036	0.018
	500 cc. dog serum			0.035	0.000
2. Horse	500 cc. egg white	0.028	0.017	0.011	0.000
3. Calf	30 cc. egg white	0.027	0.028	0.050	0.012
4. Calf	30 cc. egg white	0.000	0.000	0.035*	0.013
5. Calf	30 cc. horse serum	0.023	0.023	0.027	0.000
6. Calf	30 cc. horse serum	0.017	0.017	0.064*	0.000

* Animals under nembutal anesthesia.

occurred blood samples were taken at frequent intervals for thirty minutes to one or more hours.

Results. *The horse.* Observations were made on two horses. The antigen employed for the first animal was dog serum and that used for the second was egg white. The intravenous administration of 100 cc. dog serum before the sensitizing injections had been given produced no reaction in the horse and no significant change in the blood histamine. The control injection of 500 cc. egg white caused a mild reaction in the second horse during which the blood histamine fell from 0.028 to 0.017 γ per cubic centimeter (table 1). Within thirty minutes all signs of the reaction were gone and the blood histamine had returned to its normal value.

Following these control tests the first horse received 45 cc. dog serum by vein on twelve consecutive days and the second horse was given alternating

daily intravenous and subcutaneous injections of 40 to 50 cc. egg white for the same period. An interval of approximately three weeks was allowed to elapse before the shock dose of the antigen was again injected. In each case symptoms indicative of anaphylaxis immediately developed.

Three reactions were followed. The first animal recovered from the effects of 100 cc. dog serum in about one hour. Three weeks later a larger dose (500 cc.) was therefore given. A more marked reaction ensued which was apparently alleviated by the intravenous injection of 1 cc. of 1:1000 adrenalin and from which the animal eventually recovered. The reaction in the horse receiving egg white was so severe that the animal died eight minutes after the injection.

The symptoms of the three reactions, although differing in severity, were basically similar. The three outstanding signs of reaction were dyspnea, increased peristalsis and sweating. Almost immediately after the injection of the antigen progressive respiratory difficulty developed. In every instance it was the dominant symptom of the reaction. Recovery was associated with its disappearance and in the fatal reaction it was the apparent cause of death. As the reaction progressed the breathing became deeper and more labored. Associated with this the blood became darker and at death was almost black. Wheezes and râles could be heard some distance from the animal. In the fatal case these eventually ceased although attempts at breathing continued. Deep labored respiratory movements gave place to forced inspiratory and expiratory efforts. The picture was that of progressive respiratory obstruction which in the fatal reaction apparently became complete. Shortly after the appearance of respiratory difficulty signs of increased peristalsis were evident. Feces, at first solid and later watery, were passed frequently. Blood in the stools was never seen. Pawing the ground with the foreleg and turning the head to the flank suggested the presence of abdominal cramps. At the height of the severe reactions sweat poured from the animal in rivulets.

The histamine content of the blood was not increased during these reactions. Quantities of histamine which could be readily estimated were present in the blood before the injection of the antigen. During the reaction the histamine concentration fell and in two instances there was no evidence of histamine in the blood although less than 0.01 γ per cubic centimeter may have been present (table 1).

Control and shock samples of blood were centrifuged for determination of the distribution of the histamine. In the centrifuged specimens of control blood the white cells formed a well-defined layer which yielded 65 to 85 per cent of the whole blood histamine. The layer of white cells in the blood taken during the reaction was reduced to a thin film which contained little or no histamine. The fall in the blood histamine during the reaction was paralleled by a reduction in the histamine content of the white cell

layer. The thin indefinite white cell layer obtained in the centrifuged shock blood suggested a disappearance of the white blood cells.

During anaphylactic shock in the dog the blood as a rule fails to clot. This did not occur in these reactions with the horse. The time required for clot formation in the shock blood was generally longer than that noted for the control samples but the technic used for determination was not designed to show small changes and the differences noted may not have exceeded the limits of error of the method.

The sheep and the goat. Attempts were made to sensitize goats to egg white and sheep to egg white or dog serum. Two sheep and two goats each received 30 to 40 cc. egg white subcutaneously for ten consecutive days. Somewhat smaller doses of dog serum were given to two sheep and the subcutaneous and intravenous routes of injection were alternated for nine consecutive days.

Following an interval of two or more weeks 50 cc. of antigen were administered intravenously. For a few minutes following the injection five of the six animals showed a slight increase in the rate and depth of respiration but the response was so mild and transitory that it could not be definitely ascribed to a hypersensitive state and the experiments were abandoned. In no instances was the histamine concentration of the blood increased.

The calf. Four Guernsey calves (all under six months of age) were used in this study. Sensitization was induced by six alternating intravenous and subcutaneous 5 cc. injections of egg white or horse serum. Two of the animals received the egg white and two the horse serum. Prior to sensitization the control intravenous injection of 30 cc. egg white or horse serum caused no reaction and no change in the blood histamine (table 1).

Five to seven weeks after the last sensitizing injection the shock dose of the antigen was again administered. The first two animals tested (one egg white and one horse serum) showed definite signs of reaction and in order that more detailed observations might be made the antigen was given to the remaining two under anesthesia.

A 10 per cent solution of ethyl alcohol containing 1 grain (0.065 gram) nembutal per cubic centimeter was given intravenously. The first injection was continued until second stage anesthesia was obtained and further quantities were added as necessity arose. A total of 16 to 20 cc. was used over a period of approximately two hours. During anesthesia the animals were kept in the upright position. Changes in carotid blood pressure were followed with a mercury manometer using heparin solution as an anticoagulant. The respiratory movements were recorded by means of a tambour connected with an air-tight portion of elastic tubing fixed around the chest.

Similar signs of reaction developed in the four calves. Immediately

following injection of the antigen the animals which had not received nembutal sank on their knees to the ground and quite definitely preferred that position to standing or walking about the room. Respiration meanwhile increased in depth and rate and at this stage rather resembled panting as seen in a dog. After some minutes (about five to ten) this subsided and it was noticed that the abdomen was becoming progressively dis-

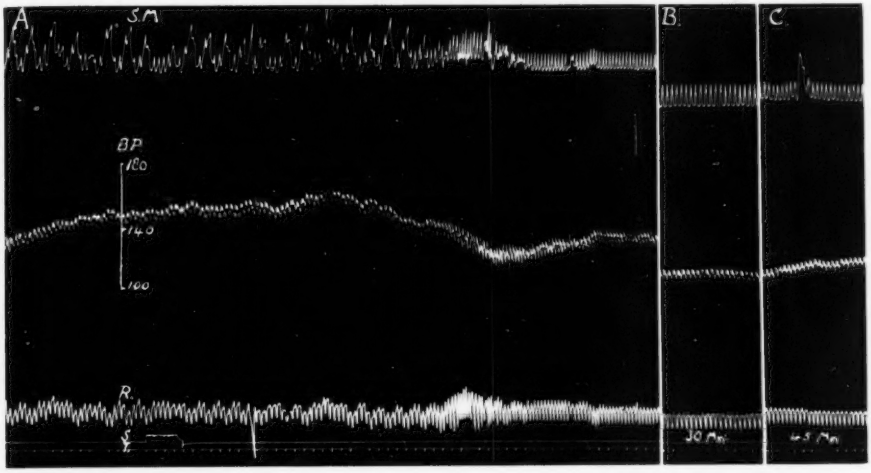

Fig. 1. The blood pressure, *B.P.*, respirations, *R*, and stomach movements, *S.M.*, before and during a mild anaphylactic reaction in a lightly anesthetized calf. The stomach movements were recorded as pressure changes in a large inflated balloon placed in the stomach and connected with a tambour.

At the signal *S* the antigen was injected intravenously. A slight gradual decline in the blood pressure occurred. For a short time the respirations were panting in character. Within three minutes the large waves characteristic of stomach contractions ceased, leaving only the small regular fluctuations due to the respiratory movements. The records were taken continuously for more than one hour. The first sign of returning gastric motility occurred forty-five minutes after the injection of the antigen, *C*. At necropsy the balloon was found in the upper anterior compartment of the stomach.

tended; indeed progressive distention of the abdomen was the most outstanding feature of the reaction. Within twenty to thirty minutes it had increased to such an extent that respiration was obviously interfered with. The abdomen was diffusely enlarged and the abdominal wall stretched taut. On percussion the note was highly tympanitic. The anesthetized animals were observed for only two hours and the outcome of the reaction in them was not noted. Both of the unanesthetized calves recovered,

although in one the abdomen was still distended six hours after injection of the antigen and the animal was ill for some days.

The calves under general anesthesia were examined when the abdomen was enormously enlarged. The cause of the enlargement was found to be distention of the stomach with gas. Deflation of the stomach restored the normal contour of the abdomen and the gas which escaped had a distinctly fermentative odor. The remaining organs appeared normal.

During the four reactions the histamine content of the blood was reduced and in two instances there was no evidence of histamine in the blood although less than 0.01 γ per cubic centimeter may have been present (table 1). No significant change was noticed in the blood clotting time. The blood pressure in the anesthetized animals tended to fall gradually during the reaction but no sudden changes were observed and the reduction which did occur may not have been specifically associated with the reaction (fig. 1).

The distention of the stomach in these animals seemed to be a definite part of the reaction. When a calf is lying on its left side the stomach is apt to become distended but the collection of gas during these experiments was quite independent of the position of the animal. The gas was apparently that which is normally formed in the stomach and its accumulation seemed due to interference with the normal process of expulsion. The distention might therefore have resulted from either spasm of the cardia and pylorus or a cessation of stomach contractions. The stomach movements during the reaction were followed in one animal under light anesthesia. A large balloon connected by pressure tubing to a recording tambour was passed down the esophagus into the stomach and then inflated under moderate pressure. The respiratory movements were accurately reflected by pressure changes in the balloon, producing small regular waves in the record, but superimposed on these were large slow fluctuations due apparently to contractions of the stomach (fig. 1). Within three minutes of injection of the antigen the stomach contraction waves ceased (fig. 1). The abdomen became progressively distended but because of the periodic escape of gas via the esophagus the distortion did not reach the extreme degree noted in other animals. The presence of the tube in the cardia evidently allowed gas to pass when sufficient pressure had become established. The experiment suggested that the distention of the stomach was due to a failure of stomach contractility during the reaction.

COMMENT

The reactions observed in the horse and calf during this study were somewhat different from those reported by Gerlach (1922) and Ritzenthaler (1924). Gerlach observed the effects in horses and cows of single and repeated injections of various serums. The outstanding features of reaction

in both species were itching, urticaria and edema of the skin with congestion and swelling of the mucous membranes. Ritzenthaler gave repeated subcutaneous and intravenous injections of egg white or beef extract to horses at intervals of about one to two weeks. Hypersensitive states were developed during which combined intravenous and subcutaneous injection of antigen produced a transitory period of dyspnea, cyanosis and increased peristalsis, followed later by a more permanent edema of the legs. Congestion with petechial hemorrhages in the mucous membranes and hemolysis of the red cells often accompanied these reactions. It seems likely from Ritzenthaler's protocols that the immediate response was due to the intravenous injection and that the changes in the skin were associated with administration by the subcutaneous route. The methods we used were designed to develop a high degree of sensitivity in response to which intravenous injection of large doses of antigen would produce acute severe anaphylactic shock. We did not study the reaction to subcutaneous injection nor did we observe the chronic effects of repeated administration of antigen. Our observations were thus limited by the technic which we adopted and it is possible that for this reason we did not observe the marked changes in the skin noticed by both Gerlach and Ritzenthaler. The difference in age between the very young calves we studied and the cows observed by Gerlach may also have been a factor in producing dissimilar reactions.

During the anaphylactic reactions which we observed in the horse and calf the histamine content of the blood was reduced. This result is in direct contrast to the marked increase in blood histamine found during anaphylactic shock in dogs and guinea pigs (Code, 1938). We have no data which offer a satisfactory explanation of this difference. It may be that anaphylaxis is not accompanied by a liberation of histamine in all species but we do not feel that our results justify this extreme interpretation. We are unable to conclude from our data that histamine was not liberated during the reactions in the horse and calf. The fate of the histamine lost from the blood during the reactions is not known. Our results simply show that histamine did not accumulate in the peripheral blood. The present understanding of the factors controlling the histamine concentration of the blood is so limited that we are unable to draw further conclusions or to suggest reliable constructive interpretations.

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THE HISTAMINE CONTENT OF THE BLOOD OF GUINEA PIGS AND DOGS DURING ANAPHYLACTIC SHOCK[1]

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That the symptoms of anaphylactic shock might be due to the formation of a chemical factor was considered early in the study of anaphylaxis (Biedl and Kraus, 1909). The similarity between the actions of histamine and the phenomena of anaphylactic shock was pointed out by Dale and Laidlaw (1910), when they described the physiologic properties of histamine. Since the publication of their studies, evidence has accumulated both for and against the elaboration of a chemical factor or, more specifically, a histamine-like substance, during anaphylactic shock. The present research was undertaken with the aim of determining any alterations in the histamine content of the blood, as estimated by the modified Barsoum and Gaddum (1935) method (Code, 1937), which might occur during anaphylactic shock in the guinea pig and the dog.

METHODS. In the majority of experiments fresh egg white or horse serum was used as the sensitizing antigen. The white of fresh eggs was diluted 1 in 5 with physiologic saline solution and thoroughly shaken until the glutinous character of the egg white disappeared. The froth was discarded and the clear solution, subsequently referred to as egg white, was used. This treatment was regarded as an essential precaution because, in an early experiment, when untreated egg white was injected intravenously symptoms simulating those of anaphylaxis developed but at necropsy the egg white was found to be lodged in the lungs.

Groups of large guinea pigs were sensitized by one or more subcutaneous injections of horse serum or egg white in doses up to 1 cc. In most instances egg white was used and dilutions up to 1 in 100 were tried. The routine finally adopted was to give 0.5 cc. of the antigen on two to four successive days. Two to four weeks after the last sensitizing injection the anaphylactic shock experiment was carried out. Control samples of blood were withdrawn by cardiac puncture and, through the same needle, the antigen was administered directly into the blood stream. The shock dose was generally 0.5 cc. horse serum or egg white. The final blood

[1] Abridgment of section of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. in Physiology.

sample was taken by transection of the blood vessels of the neck. The thoracic contents were always examined to check the presence or absence of true anaphylactic shock.

Dogs were sensitized by nine to fourteen daily intravenous or alternating intravenous and subcutaneous injections of 0.5 cc. horse serum or egg white per kilogram of body weight. The shock dose in both cases was 15 to 30 cc. (2 to 4 cc. per kilogram of body weight) and this was given intravenously fourteen to 210 days after the last sensitizing injection.

Dr. J. L. Bollman very kindly placed at my disposal three dogs which he had sensitized to duck serum. The results obtained with these animals are separately given in one of the tables which follow.

The shock dose of antigen was given to three dogs under general anesthesia, obtained by the intravenous injection of approximately 25 mgm. nembutal or 50 mgm. sodium amytal per kilogram of body weight. During four reactions the blood pressure was recorded by a mercury manometer connected with a cannula placed in the femoral or carotid artery. In each experiment with the dog the clotting time of the blood was roughly estimated by placing approximately 1 cc. blood in a small clean test tube (capacity 4 cc.) and noting the time taken for the blood to cease flowing on inversion of the tube.

Extracts of blood for histamine determination were prepared by the modified Barsoum-Gaddum method (Code, 1937). Owing to the small quantity of blood obtainable from guinea pigs, duplicate sampling was seldom possible and extracts were at times prepared from as little as 3 cc. With the dog, extracts were made from 5 cc. of blood and in these experiments duplicate extracts were always prepared during the control period, but throughout the reaction when samples were taken at frequent intervals and conservation of the circulating blood was desired, the duplication was often omitted. The technic used to determine the distribution of histamine in the blood by separating the plasma and cellular components has been described in detail (Code, 1937). In most instances, since the shock blood did not clot no anticoagulant was used. When prevention of clotting was indicated heparin solution (Connaught Laboratories) was added to the blood. The histamine content of the extracts was estimated as a routine on the lower part of the ileum of the guinea pig suspended in 2 to 3 cc. Tyrode's solution containing 1.0 γ atropine sulfate per cubic centimeter. All histamine determinations were expressed in terms of histamine base. As previously reported, histamine equivalent values of approximately 0.01 γ per cubic centimeter of blood cannot be consistently assayed nor can activity of this order be definitely ascribed to the presence of histamine in the blood. For these reasons all values below 0.01 γ per cubic centimeter were regarded as subliminal in activity and are recorded as 0.000 γ per cubic centimeter.

RESULTS. *Anaphylactic shock in the guinea pig.* The histamine content of control samples of blood taken from normal and sensitized guinea pigs was quite constant. The cardiac blood of twenty unselected animals had a mean histamine content of 0.133 γ per cubic centimeter $\pm$ 0.007. The variability of these estimations measured as standard deviation was 0.047 γ per cubic centimeter and the maximal and minimal concentrations were 0.280 and 0.066 γ per cubic centimeter.

Animals in which such severe anaphylactic shock had developed that recovery seemed impossible when the second blood sample was taken showed an increase in the histamine content of the blood from two to thirteen times the control value (table 1). These animals suffered extreme respiratory difficulty and at necropsy the voluminous lungs typical of anaphylactic shock were always found. Milder grades of anaphylaxis

TABLE 1

The histamine content of the blood of guinea pigs before and during severe anaphylactic shock and nitrogen anoxemia

GUINEA PIG	CONDITION	γ HISTAMINE PER CC. BLOOD	
		Before	During
1	Anaphylactic shock	0.150	0.600
2	Anaphylactic shock	0.127	0.959
3	Anaphylactic shock	0.145	0.444
4	Anaphylactic shock	0.166	1.250
5	Anaphylactic shock	0.080	0.779
6	Anaphylactic shock	0.184	0.685
7	Anaphylactic shock	0.075	0.358
8	Anaphylactic shock	0.066	0.917
9	Nitrogen anoxemia	0.143	0.118
10	Nitrogen anoxemia	0.149	0.141
11	Nitrogen anoxemia	0.118	0.066
12	Nitrogen anoxemia	0.091	0.160

gave somewhat variable results. Animals having definite, though not fatal, respiratory difficulty generally showed slight increases in the blood histamine, for example, 0.141 to 0.259 γ per cubic centimeter. When the animal merely scratched its nose and gave a few coughs, no increase in blood histamine was observed. Likewise the blood histamine of unsensitized control animals was not elevated by administration of the antigen. The increased histamine content of the blood during severe anaphylaxis was not due to the technic employed in procuring the blood samples.

Barsoum and Gaddum (1935) first demonstrated an increase in the histamine equivalent of blood from a limb during vascular occlusion. The guinea pig in severe anaphylactic shock suffers from an extreme degree of oxygen lack and it seemed possible that this alone might account for

the increase in blood histamine during the reaction. Four animals were separately placed beneath an inverted jar in which the air was replaced by pure nitrogen. Blood samples were taken as in the anaphylactic experiments, before exposure to nitrogen and during respiratory failure. In three of these animals, respiration had ceased although the heart was beating forcefully when the second blood sample was taken. The blood histamine was, if anything, reduced by the anoxemia (guinea pigs 9, 10 and 11, table 1). The last animal of the series was removed from the nitrogen chamber before respiratory failure set in and the histamine was found to have increased from 0.09 to 0.16 γ per cubic centimeter of blood, an increase considerably less than that occurring in anaphylaxis. Conditions obtaining with the first three animals of the series corresponded more closely to those of the anaphylactic experiments and it seemed permissible to conclude that the increased quantity of histamine found in the blood during anaphylactic shock was not due to the coincident anoxemia.

An indication that factors in addition to anoxemia were present during severe anaphylaxis was also noticed when the quantity of blood obtained by transection of the vessels of the neck during anaphylactic shock was compared with that given during nitrogen anoxemia. While it was often difficult to obtain a single 4 to 5 cc. blood sample from guinea pigs in anaphylactic shock, those suffering from nitrogen anoxemia rapidly yielded 10 cc. or more.

The experiments allowed the conclusion that, in severe anaphylactic shock in the guinea pig, the histamine content of the blood is increased and that this increase is not due to the coincident anoxemia.

Anaphylactic shock in the dog. The anaphylactic reactions of dogs sensitized to egg white and horse serum were controlled by intravenous administration of the shock dose of the antigen before the sensitizing injections were commenced. Egg white caused no reaction in the unsensitized dogs and no significant alteration in the histamine content of the blood (table 2). In two animals, injection of horse serum produced a mild reaction during which feces were passed and the blood became dark although it clotted in the usual time. Coincident with these mild reactions there was a slight rise in blood histamine (table 2, dogs 7 and 9).

Two or more weeks after sensitization the shock dose of the antigen was again administered. Whenever typical signs of anaphylactic shock developed the histamine content of the blood was increased from two to more than eighty times the control value (table 3).

The animals sensitized to duck serum had received their preliminary injections some months before this study was started and the control effects of the duck serum had not been tested. One animal was therefore desensitized by small subcutaneous and intravenous injections, following which the duck serum given intravenously caused no reaction and no

alteration in the blood histamine. Later, with return of sensitivity, duck serum injection evoked anaphylactic shock accompanied by a marked elevation in the histamine content of the blood (table 3, animal 13).

TABLE 2

The histamine content of the blood of normal dogs before and after the administration of egg white and horse serum

DOG	ANTIGEN	REACTION	γ HISTAMINE PER CC. BLOOD	
			Before injection of antigen	Maximum after injection of antigen
1	Egg white	Nil	0.023	0.020
2	Egg white	Nil	0.021	0.015
3	Egg white	Nil	0.000	0.010
4	Egg white	Nil	0.000	0.000
5	Egg white	Nil	0.029	0.029
6	Egg white	Nil	0.000	0.000
7	Horse serum	Mild	0.037	0.064
8	Horse serum	Nil	0.025	0.022
9	Horse serum	Mild	0.000	0.032
10	Horse serum	Nil	0.020	0.000

TABLE 3

The histamine content of the blood of sensitized dogs before and during anaphylactic shock

DOG	ANTIGEN	γ HISTAMINE PER CC. BLOOD	
		Before injection of antigen	Maximum after injection of antigen
1	Egg white	0.000	0.285
2	Egg white	0.000	0.286
3*	Egg white	0.010	0.118
4	Egg white	0.014	1.250
7	Horse serum	0.023	0.200
8*	Horse serum	0.010	0.500
9*	Horse serum	0.000	1.335
10	Horse serum	0.040	0.121
11	Duck serum	0.015	0.949
12	Duck serum	0.023	0.228
13	Duck serum		
	Desensitized	0.024	0.021
	Sensitized	0.036	0.435

* Animals under general anesthesia.

In three experiments shock was induced under general anesthesia (table 3, dogs 3, 8 and 9). Except that vomiting did not occur, the reaction and the behavior of the blood histamine seemed the same in the anesthetized and unanesthetized dogs.

Throughout these experiments a convenient indicator of the presence of anaphylactic shock was the failure of the blood to clot. In every instance of anaphylaxis in the dog in which the blood did not clot there was a marked increase in the blood histamine. In conjunction with other studies these results have now been confirmed in more than twenty such reactions and it seems permissible to conclude that in the dog anaphylactic shock is associated with an increase in the histamine content of the blood.

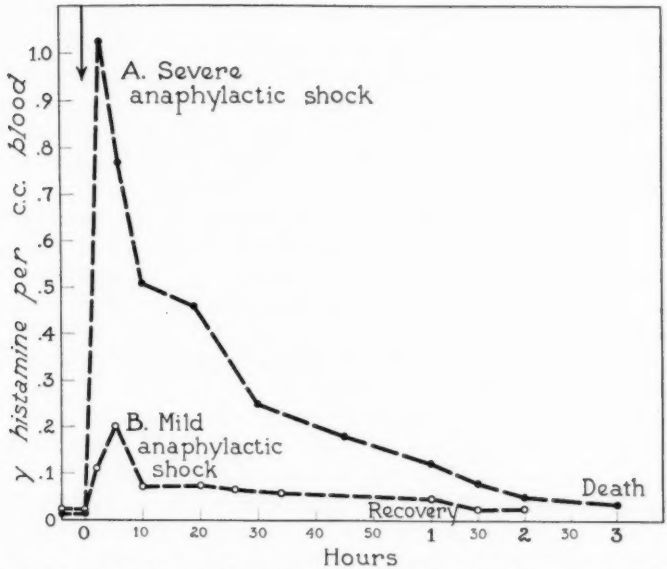

Fig. 1. Blood histamine changes during anaphylactic shock in unanesthetized dogs. At the arrow the antigen was given intravenously. A. Severe reaction; by twenty minutes the animal was deep in shock; intravenous fluids and adrenalin were given but despite these death occurred just after the three hour mark. B. Mild reaction; the animal recovered completely within two hours.

Experiments were next carried out to determine, if possible, the nature and the behavior of the increase of blood histamine during anaphylaxis and to gain insight into the possible rôle of histamine in the anaphylactic reaction.

The blood histamine level during the reaction. In most experiments, after the injection of the antigen, the histamine content of the blood was followed at intervals for one, two, three or more hours. It was found that if determinations were to be made when the blood histamine was at its maximum, samples had to be taken frequently during the first few minutes of the reaction (fig. 1). With one exception, the maximal increase in the

blood histamine was reached in three to ten minutes after injection of the antigen (exception, seventeen minutes). Following this early peak the histamine content fell rapidly, often returning to its normal level in two or three hours (fig. 1). There is, apparently, an explosive liberation of histamine coincident with the dissemination of the antigen throughout the body.

The distribution of the histamine in the blood. The major part of the histamine in normal unclotted blood is held within the white cells and is not free to produce its physiologic effects (Code, 1936, 1937). Before the significance of the increased quantity of histamine in the blood during anaphylaxis could be appraised it was necessary to determine quantitatively its distribution between the plasma and cellular components of the blood. Blood samples of 20 to 50 cc. were taken for centrifugation—before and after administration of the antigen. In most instances the shock blood was withdrawn during the first seven minutes of the reaction in order to ascertain the distribution at the peak of the histamine rise. As in earlier reports, results were expressed in terms of γ histamine distribution in the components of 10 cc. blood. As an indicator of the accuracy of the methods involved, the histamine values for the separated parts of the blood were added together and this calculated total for 10 cc. blood compared with the estimated histamine content of 10 cc. of the same blood uncentrifuged (table 4).

In the blood withdrawn during anaphylactic shock the white cell layer was greatly reduced, being generally represented by an indefinite film in contrast to the clear-cut layer found in the control samples. During the reaction most of the white cells had disappeared from the blood and what histamine the white cell layer was estimated to contain was probably due to contamination with plasma containing histamine. Some of the histamine was found in the red cells but its major source was the plasma (table 4). If the blood was centrifuged at the time of the explosive liberation of histamine 79 to 91 per cent of the whole blood histamine was found in the plasma.

In two experiments the distribution was determined when the peak of the histamine rise had passed and the concentration was returning to normal. In the first experiment, twenty minutes after administration of the antigen the histamine concentration of the whole blood had fallen from a maximum of 0.200 to 0.077 γ per cubic centimeter and 64 per cent of this was contained in the red cells. The reaction in the second experiment was more severe and for purposes of comparison the distribution was determined in blood taken seven minutes and one hour after injection of the antigen. In the first seven minutes the blood histamine had risen to more than 1 γ per cubic centimeter and 80 per cent was present in the plasma. In the one hour sample the histamine concentration was 0.448 γ

per cubic centimeter of which 41 per cent was found in the plasma and 58 per cent in the red cells. During this interval the histamine content of the red cells had not significantly altered (0.400 and 0.444 γ per cubic centimeter at seven minutes and one hour respectively) but the plasma

TABLE 4

The distribution of histamine in blood taken before and during anaphylactic shock in the dog

DOG	COMPONENTS OF BLOOD	γ HISTAMINE IN STATED COMPONENT OF 10 CC. BLOOD		PER CENT DISTRIBUTION OF HISTAMINE IN SHOCK BLOOD
		Control; before injection of antigen	Shock; 3 to 7 min. after injection of antigen	
A	Plasma	0.05	10.39	83
	White cell layer	0.00	0.29	2
	Red cells	0.00	1.88	15
	Calculated whole blood	0.05	12.56	
	Estimated whole blood	0.00	13.35	
B	Plasma	0.00	2.90	79
	White cell layer	0.07	0.42	11
	Red cells	0.00	0.35	10
	Calculated whole blood	0.07	3.67	
	Estimated whole blood	0.10	3.35	
C	Plasma	0.00	1.12	91
	White cell layer	0.00	0.02	2
	Red cells	0.00	0.09	7
	Calculated whole blood	0.00	1.23	
	Estimated whole blood	0.00	1.18	

Explanatory note. A volume of 10 cc. whole blood was chosen as a unit for the expression of histamine distribution. The concentration of histamine in plasma and red cells, separated by centrifugation, was determined. The quantity of histamine contained in the plasma and red cells of 10 cc. blood was calculated from these values and hematocrit determinations. The white cell layer from 20 to 50 cc. centrifuged blood was collected and its histamine content determined and from this was calculated the amount of histamine contained in the white cell layer from 10 cc. blood. By adding the amounts of histamine contained in the separated parts of the blood the calculated whole blood histamine content was obtained. As an indication of the accuracy of the procedures involved this calculated total was compared with the histamine content of 10 cc. of the same blood estimated without centrifuging.

values had fallen from 1.961 to 0.308 γ per cubic centimeter. In this and other experiments, the red cells finally lost their histamine, for the whole blood concentration eventually returned to normal or approximately normal values. The data suggest initial liberation of histamine into the fluid portion of the blood with some diffusion into the red cells,

followed by a rapid destruction or disappearance of the plasma histamine and a more gradual decline in the red cell concentration.

The state of the plasma histamine. As estimated by the modified method the histamine content of the plasma was high during the initial stages of anaphylaxis. There remained the possibility, however, that the histamine estimated to be present in the plasma was not "free" and that the chemical procedures involved in extraction contributed to the activity. Plasma separated at the time of maximal histamine output and tested without chemical treatment was found to be highly active. The active substance was also present in simple trichloracetic acid filtrates of plasma. In two experiments quantitative comparison was made of the activity found in samples of untreated plasma, neutralized trichloracetic acid filtrates of plasma and extracts of plasma prepared by the modified process. The values obtained agreed within 15 per cent. The chemical methods used were not responsible for the estimated activity and the histamine found in the plasma during anaphylactic shock was not bound in any way but was free to produce its physiologic effects.

The blood pressure and the blood histamine. During anaphylactic shock in four animals, the blood pressure was recorded and the blood histamine frequently determined. In three instances general anesthesia was employed and in one instance local infiltration with 2 per cent procaine (without adrenalin) enabled the femoral blood pressure to be taken during the reaction. Heparin in physiologic saline solution was used to prevent clotting in the blood pressure tubing.

In every experiment the precipitous fall in blood pressure during the early stages of the reaction was simultaneous with the rapid accumulation of histamine in the blood (fig. 2). The duration of the lowered blood pressure was related to the concentration of histamine attained in the blood and the time required for the excess histamine to disappear. When the reaction was mild the blood histamine rose during the first few minutes to between 0.1 and 0.2 γ per cubic centimeter and at the same time the blood pressure fell to almost zero, but within an hour the histamine returned to its control value and the pressure rose to approximately its normal level (fig. 2A). More severe reactions caused greater and more prolonged increases in the histamine content of the blood. At the end of one hour the blood histamine was still elevated and the blood pressure still far below normal (fig. 2B). In the most severe reaction of these four experiments the histamine concentration rose to more than 1 γ per cubic centimeter and continued above normal for more than two hours. The blood pressure did not recover and despite supporting measures the animal died with an elevated blood histamine and a lowered blood pressure (fig. 2C).

The fall in blood pressure in anaphylactic shock was coincident with the

explosive liberation of histamine and recovery of the blood pressure occurred only when the histamine disappeared from the blood. It seemed

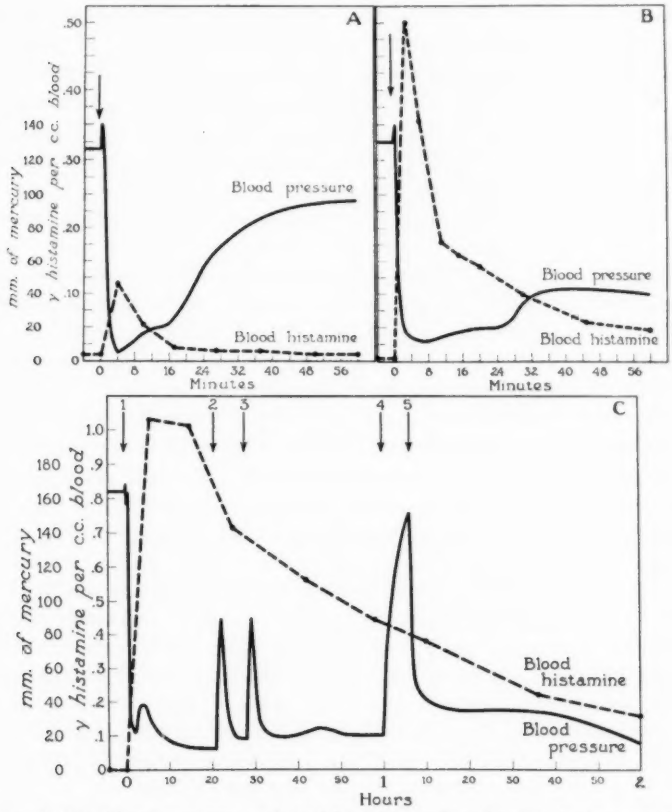

Fig. 2. The blood pressure and the blood histamine changes during anaphylactic shock in anesthetized dogs. The solid line is charted from a continuous record of the arterial blood pressure. Points joined by the interrupted line show the concentration of histamine in the blood. At the first arrow in each experiment the antigen was injected intravenously. A, mild anaphylactic shock. B, a more marked reaction. C, severe anaphylactic shock. Seventeen minutes after injection of antigen animal ceased breathing and pulse could not be felt. Artificial respiration was commenced and at arrows 2 and 3, 0.5 cc. 1 to 1000 adrenalin was given. Between arrows 4 and 5, 0.8 cc. adrenalin in 180 cc. 10 per cent solution of glucose was injected intravenously. Despite these supporting measures animal died a few minutes after the two-hour mark.

likely that a rapid intravenous injection of histamine would reproduce somewhat parallel changes and by comparison assist in appraising the

significance of the blood histamine rise in anaphylactic shock. Four experiments were carried out in which histamine was given to normal dogs in the amount calculated to be necessary to raise the concentration in the total fluid available for the dilution of diffusible substances in the body of the animal to between 0.4 and 0.7 γ per cubic centimeter. The quantity of fluid diluting the histamine in the animal was taken as 60 per cent of the body weight. On the basis of this calculation, dogs weighing approximately 7 and 14 kgm. were given 2 to 5 mgm. histamine base by vein during a two to three minute period. Commencing one minute after injection, samples of blood for histamine determination were taken at intervals for one or more hours. During two experiments the blood pressure was recorded.

The highest histamine values always occurred in the blood drawn one minute after injection and the amount present at that time approached the calculated concentration (0.2 to 1.1 γ per cubic centimeter). Administration on different occasions of 2 and 3 mgm. histamine base to a 7 kgm. dog raised the venous blood histamine in one minute to 0.2 and 0.4 γ per cubic centimeter and at the same time lowered the blood pressure to 24 and 9 mm. of mercury respectively. Following this low point the blood pressure rapidly recovered and had returned to an approximately normal level within twenty minutes. This prompt recovery of the blood pressure was associated with an equally rapid disappearance of the histamine from the blood. In every experiment the histamine had practically gone from the blood within thirty minutes and at the end of one hour the concentration was always less than 0.05 γ per cubic centimeter. The decline in the histamine content of the blood was more rapid than that observed during anaphylactic reactions. This difference was particularly marked when the histamine concentration rose to approximately 1 γ per cubic centimeter of blood; at the end of one hour following injection it had practically disappeared from the blood, but during anaphylaxis the concentration was stiil more than 0.1 γ per cubic centimeter.

In both the anaphylactic reactions and the histamine injection experiments recovery of the blood pressure was associated with disappearance of the histamine from the blood. The more rapid return to normal pressures following injection was associated with a more rapid disappearance of histamine. The time required for the blood histamine to reach its maximum after administration of the antigen in anaphylactic shock was always more than three minutes. The time devoted to injection of histamine in normal dogs never exceeded three minutes. The experiments indicated that larger doses and more prolonged injections of histamine than those used would be required to elevate the blood histamine to a maximum during the first ten minutes and to reproduce the declining concentration as found in anaphylactic shock.

The severity of the reaction and the concentration of histamine in the blood. No special criteria were formulated to grade the degree of anaphylactic shock. A simple means of classification was found to be the final outcome of the reaction and on this basis a comparison was made between the histamine content of the blood and the severity of the reaction. Certain difficulties in the appraisal of this comparison must be pointed out. The maximal histamine concentration recorded during a reaction was represented by a single sample of blood taken at a time when rapid changes were occurring in the histamine level. In some experiments it seemed clear that the peak histamine value had been missed (fig. 2C) and, in other instances, the maximal value recorded may not represent the height actually attained in the blood. During the early stages of the most severe reactions, supporting measures (adrenalin, transfusion and artificial respiration) were often administered and it seemed certain that these did delay the fatal outcome (fig. 2C). Death in the first few minutes of a reaction, when the blood histamine was high, was thus at times postponed by treatment until the histamine concentration had returned to approximately normal figures. Despite these limitations, comparison on broad lines indicated a relationship between the histamine content of the blood and the degree of anaphylactic shock.

In mild reactions only small quantities of histamine were found in the blood (up to approximately 0.2 γ per cubic centimeter) and recovery of the animal was complete in one or two hours. During more severe anaphylaxis histamine was found in greater concentrations (maximum about 0.5 γ per cubic centimeter of blood, fig. 2B) but as a rule it had disappeared from the blood at the end of two or three hours. Despite the return of the blood histamine to normal values many of these animals died six to twelve hours after the onset of the reaction. Examination revealed the signs of obstruction to the portal circulation which had been manifested before death by a bloody diarrhea. In only the most severe and rapidly fatal reactions could death be directly attributed to the liberation of histamine. In such a reaction the blood histamine rose rapidly to more than 1 γ per cubic centimeter and remained elevated until death resulted from circulatory failure.

Comment. Throughout this investigation the histamine equivalent of whole blood was estimated as a routine by the modified Barsoum-Gaddum method. When Code and Ing (1937) isolated the active substance estimated by this procedure and showed it to be histamine, they stressed the limitation of their findings to blood from normal animals and suggested certain errors which might arise in accepting, without further evidence, results obtained during abnormal conditions. In their observations, active substances other than histamine did not survive the chemical extraction nor did the process of extraction itself produce activity, yet

they felt that such possibilities should be considered. In normal blood the source of the histamine was found to be the leukocytes, but that this might not be true in abnormal conditions was clearly pointed out.

The quantity of blood obtained from guinea pigs during anaphylactic shock was so small that the source and nature of the increased activity were not studied. Plasma separated during anaphylactic shock in the dog contained the active ingredient and its concentration was the same in samples of plasma estimated without chemical treatment, after removal of protein by trichloracetic acid and following complete extraction by the modified method. The active ingredient was not produced by the chemical procedures involved in extraction. The results of these tests are similar to those observed by Code and Ing. They indicate that histamine alone was being estimated.

When calculating the dose of histamine necessary to raise the blood histamine level in the dog, dilution with fluid representing 60 per cent of the body weight was considered. This percentage is an approximate average adopted from studies in which sodium chloride and sucrose were injected intravenously into dogs and the dilution in the body determined (Baldes, Johnson, Seeley and Essex). The diffusibility of histamine may not be the same as that of sodium chloride or sucrose and the figure used when applied to histamine may be far from correct. Its use, however, seemed justified since it avoided the obvious error of calculating the dose on the basis of blood volume alone and indicated that dilution outside the vascular tree was at least being considered. The concentration in blood drawn one minute after injection approached the calculated level but sufficient time for dissemination throughout the body could hardly have elapsed. The rapid disappearance of histamine from the blood, without establishment for even a short time of a constant level, indicated that equilibrium throughout the body was probably never attained and that active destruction of histamine was in progress. The fall in the blood histamine during anaphylactic shock, although slower, showed the same general character and may also be interpreted as indicating destruction of the histamine liberated during the reaction. These results are in accord with the earlier studies of Best and McHenry (1930) on the inactivation of histamine by the tissues.

The accumulated evidence shows that histamine is liberated during anaphylactic shock in the guinea pig and dog and that it plays a rôle in the symptomatology of the reaction in these species. Bartosch, Feldberg and Nagel (1932) found that a histamine-like substance was liberated into the perfusate when the antigen was administered to the isolated perfused lungs of sensitized guinea pigs. Schild (1937) extended these observations to other organs of the guinea pig and obtained positive results in the majority of tissues tested. These findings suggest that in the present

study on the intact guinea pig the increase in the blood histamine during severe anaphylactic shock was due to its liberation from the tissues of the animal. In four of the eight severe reactions encountered the blood histamine rose to approximately 1 γ per cubic centimeter. If this concentration were attained in fluid representing approximately 60 per cent of the body weight of an 800 to 1000 gram guinea pig, a total of 0.5 mgm. would be present. This is the intravenous dose of histamine which Dale and Laidlaw (1910) found to be fatal for 800 to 1000 gram guinea pigs.

The cross circulation and liver exclusion experiments of Manwaring (1910) indicated the liberation of a depressor substance by the liver during anaphylactic shock in the dog. Dragstedt and Gebauer-Fuelnegg (1932) demonstrated the presence of histamine activity in the blood of the supradiaphragmatic inferior vena cava and the lymph of the thoracic duct during canine anaphylaxis. They occasionally noted activity in blood taken from the femoral vein. In the present investigation the use of a more sensitive procedure for detection accounts for the consistent demonstration of increased quantities of histamine in the peripheral blood even during mild reactions.

In the studies by Weil (1917) blood was taken from the carotid arteries of dogs so deep in anaphylactic shock that death was imminent and this was given intravenously to normal dogs under ether anesthesia. Following recovery from the anesthesia no signs of anaphylaxis were seen. Blood pressure records were not reported. Weil used highly sensitized animals which regularly died one-half to one hour after administration of the antigen. Even in these animals most of the histamine liberated during the early minutes of the reaction would have disappeared from the blood by the time death became imminent. The condition of the test animals after recovery from anesthesia would not reveal the presence of the relatively small quantity of histamine in the transfused blood.

The amount of histamine found in the peripheral blood is not large and, as indicated by the histamine injection experiments, represents only a small portion of that liberated. Manwaring, Hosepian, O'Neill and Moy (1925) observed indefinite responses in partially exsanguinated normal dogs on the injection of carotid artery blood taken from dogs during the early stages of anaphylactic shock. The greatest concentration of histamine ever found in the blood in the present study was approximately 1 γ per cubic centimeter. The normal dog would withstand the injection of large quantities of such blood and relatively enormous volumes would be necessary to reproduce anything like the prolonged fall in blood pressure observed during anaphylactic shock.

The blood pressure changes during the early stages of anaphylactic shock in the dog are related to the concentration of histamine in the blood. The rapid rise in the histamine content is simultaneous with the sudden

fall in blood pressure and the disappearance of the histamine is accompanied by recovery of the blood pressure. A comparison of the blood histamine values following the injection of known amounts of histamine with those occurring during anaphylactic shock indicates that sufficient histamine is liberated in anaphylaxis to produce the precipitous fall in blood pressure. In severe reactions the circulation is flooded with histamine in the face of which the blood pressure cannot recover and the animal quickly succumbs to the effects of a lowered blood pressure. The complete picture of anaphylactic shock in the dog is not, however, explained on the basis of the general effects of histamine. Important alterations occur in the liver, the cause and consequences of which are not clearly understood, and the extent to which they may contribute to the fall in blood pressure was not appraised in this study.

The liberation of histamine during anaphylactic shock in the guinea pig and the dog does not establish it as a fundamental accompaniment of anaphylaxis. Extension of the observations to other species has given significantly different results.

SUMMARY

The changes in the histamine content of the blood, as estimated by the modified Barsoum-Gaddum method, were determined during anaphylactic shock in guinea pigs and dogs. During severe anaphylactic shock in guinea pigs the blood histamine was increased from two to thirteen times the control value. The results of experiments in which oxygen lack was induced by nitrogen administration showed that the increased quantity of histamine found in the blood during severe reactions was not due to the coincident anoxemia. In dogs showing typical signs of anaphylactic shock, the histamine content of the blood was increased from two to more than eighty times the control value. The maximal concentration was generally reached during the first ten minutes of the reaction, following which the histamine content fell rapidly and often returned to normal within two or three hours. During the time of maximal concentration 79 to 91 per cent of the whole blood histamine was found free in the plasma.

The fall in the blood pressure which occurs in canine anaphylaxis was coincident with the rapid accumulation of histamine in the blood and recovery of the blood pressure occurred only when the histamine disappeared from the blood. Experiments were carried out in an attempt to appraise the significance of these findings. The data offered an explanation for the failure of some investigators to obtain evidence indicating the liberation of a toxic factor during anaphylactic shock in the dog.

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RESPIRATORY AND VASOMOTOR EFFECTS OF VARIATIONS IN CAROTID BODY TEMPERATURE[1]

A Study of the Mechanism of Chemoceptor Stimulation[2]

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The chemoceptors in the carotid body are capable of responding to a great variety of chemical changes occurring in their surroundings. A matter of great interest is the nature of the chemical changes which occur *within* the chemoceptors when they are exposed to chemical agencies and which result in their varying their rate of discharge. A working hypothesis (Winder, 1937; Bernthal, 1938) extends to the chemoreceptors the principles advanced by Gesell (1925; 1929) for the chemical control of the respiratory and vasomotor centers. According to this conception, a factor which determines the degree of functional activity of the chemoceptors is their own intracellular acidity which is dependent upon the rate and quality of their own acid metabolism as well as upon the properties of their environment.

To test this hypothesis directly would require measurement of changes in metabolite concentration within the chemoreceptor cell. In the absence of methods for accomplishing a direct test, recourse must be had to indirect evidence and there is already a certain amount of this which appears to support the hypothesis. Thus, the use of iodoacetic acid to prevent metabolic formation of lactic acid during anoxia prevents the response of the chemoceptors to anoxia (Winder, 1937), a finding which suggests a causal relationship between intracellular acidity and response. Such a relationship is further suggested by the finding that changes in chemoceptor activity, as they are reflected in vasomotor and respiratory chemoreflex reactions (Bernthal, 1932, 1934, 1938), run parallel to theoretically concurrent changes in their own acidity. Further, there is the observation that curtailed blood supply of the carotid body, a factor predisposing to the intracellular accumulation of the (acid) products of both anaerobic and aerobic metabolism, leads to an increase in chemoceptor discharge

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(Samaan and Stella, 1935) and in the reflex respiratory and vasomotor reactions which are dependent upon such discharge (Winder, Bernthal and Weeks, 1938).

Adequate testing of an hypothesis by indirect evidence alone is dependent in the final analysis upon variety and sheer quantity of such evidence and further tests are obviously necessary in the present instance. Varying the temperature of the carotid body provides such a test, for variations of temperature with their attendant variations in rate of metabolism and metabolite formation must produce variations in intracellular acidity. A finding of increased chemoceptor discharge with increased temperature would be in harmony with the hypothesis and might be considered to afford it indirect support. On the other hand, if opposite effects were found or if temperature variations had no effect upon carotid body discharge, the finding might constitute a grave obstacle to acceptance of the hypothesis.

Zbyszewski (1928) and Tournade and Dubreuil (1932) observed that warming the carotid bifurcation produced arterial hypotension and this, if it arises reflexly from the carotid body, indicates *decreased* chemoceptor activity. Likewise, Tournade, Malmejac and Jourdan (1929) report that increased temperature of fluid perfusing the carotid region results in peripheral vascular dilatation (of the paw). However, coupled with this apparent evidence of decreased chemoceptor discharge, the latter authors report an accelerated breathing which, if it arises from the carotid body, is evidence of *increased* chemoceptor activity. It is extremely improbable that these two reactions could arise simultaneously from the carotid body and their occurrence together points to some structure other than or in addition to the carotid body as the source. The similarity of the phenomenon described by Tournade et al. to the long established effects of allowing warmed carotid blood to reach the brain (Kahn, 1904; Moorhouse, 1912) points to the latter as a factor in determining the results and suggests as well that the original findings of Zbyszewski might be explained upon this basis. It is obvious that warming of the brain would inevitably result from warming of the carotid blood unless strict precautions for adequate vascular isolation of the carotid bifurcation were observed.

Because of these uncertainties concerning the true source of the reactions already described in the literature, it was necessary to devise new experiments in which specific precautions were exercised toward limiting the changes in temperature to the region of the carotid body alone.

Method. In heparinized dogs narcotized with morphine and urethane or with morphine and chloralose, that portion of each carotid region which supplied the carotid body was perfused with arterial blood at temperatures varying between 15°C. and 45°C. Reflex respiratory reactions which resulted from the changing temperatures at the carotid body were recorded

by a sensitive spirometer and reflex vasomotor reactions were indicated by changes in volume flow of blood in one of the axillary arteries (thermoelectric method), all other arteries communicating with the foreleg having first been ligated.

The blood perfusing the axillary artery and the carotid region was supplied at an evenly pulsating pressure having a constant mean value. The equipment used for perfusion was that described in connection with another investigation (Bernthal, 1938). The fraction of the perfusing

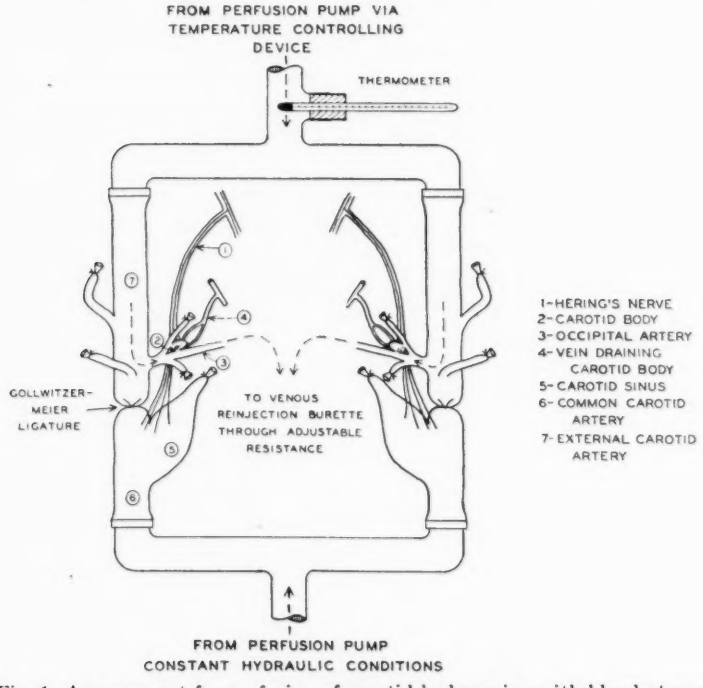

Fig. 1. Arrangement for perfusion of carotid body region with blood at varying temperatures and for segregation of pressoreceptor and chemoreceptor zones.

blood destined for the carotid body was led through a simple temperature adjusting device consisting of about 70 cm. of silver tubing enclosed in a water jacket containing flowing water the temperature of which could be accurately controlled at various levels.

The blood from the temperature controlling device was circulated through the external carotid and occipital arteries as indicated in figure 1. The ligatures placed upon the external carotid arteries after the manner of Gollwitzer-Meier (1934) and of Comroe and Schmidt (1938), served the

purpose of excluding the carotid sinus pressoreceptor zones from the temperature variations. Constant intensity of the stream of tonic impulses normally leaving these zones was encouraged by perfusing them from a separate pump outlet under constant conditions and at a mean pressure corresponding to that of the animal.

To accomplish the primary object of these experiments, it was necessary to exclude blood of varying temperature not only from pressoreceptors but from all structures peripheral to the carotid bifurcation. It is to be emphasized that the results which we report cannot be duplicated in most individuals if warmed or cooled blood is allowed to reach the intracranial circulation even in very small quantities. By the use of transillumination to aid in visualizing the smaller vessels and by the judicious placing of mass ligatures where necessary, complete vascular isolation of the carotid bifurcation can usually be accomplished. The test of the adequacy of such isolation is simple and conclusive and was routinely applied, namely, the sectioning of Hering's nerves at the completion of the experiments. Satisfactory isolation left no residual effects of temperature variations after the cutting of the nerves.

Results. The reactions of 19 experimental animals provide the basis for this report. The total number of experiments performed was considerably greater than this but a certain number were barred from consideration by unsatisfactory control procedures (persistence of reactions after the sectioning of Hering's nerves or the appearance of red lead in the cerebral circulation when postmortem injections were made under pressure into the supposedly isolated carotid bifurcation). In 10 of the 19 satisfactorily controlled experiments, vasomotor studies were made.

Several examples of reflex respiratory reactions to changing carotid body temperatures are reproduced in figure 2 and are sufficiently clear to require little further description. The minute volume of breathing is diminished when carotid body temperature is diminished below normal (body temperature) and is increased when carotid body temperature is increased above normal. The maximum percentage diminution in minute volume recorded upon cooling from body temperature was 34 per cent and the maximum increase upon warming from body temperature was 32.6 per cent. The percentage increase above normal was much more marked than this (65–75 per cent) if the temperature of the blood was changed directly from cool to warm. This point is illustrated by figures 2C and 2D which were taken from closely consecutive portions of the same experiment. It can be seen that approximately the same degree of carotid body hyperthermia produced a minute volume of 5.50 liters (19.1 per cent above normal) when preceded by body temperature and a minute volume of 6.53 liters (41.3 per cent above normal) when preceded by cold. The obvious explanation is that the exaggerated response includes not only the direct

effects of hyperthermia but also a compensatory reaction for the hypoventilation which resulted from the preceding cold. That there may be additional factors causing the enhanced response is suggested by the fact that it is seen even in rare experiments in which the hypoventilation due to cold is very small. In figure 2D, for example, the 5 per cent hypo-

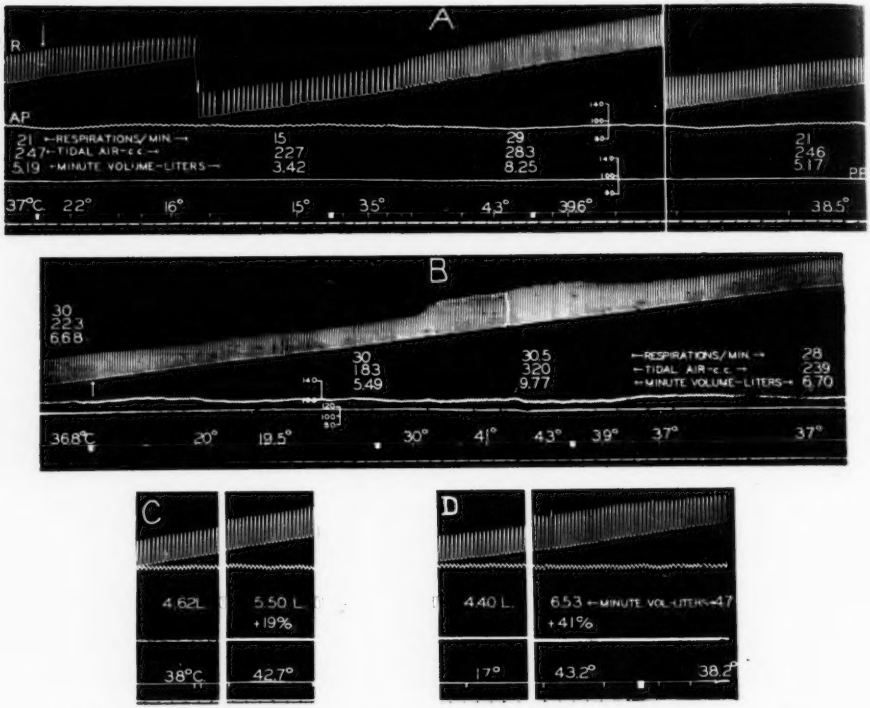

Fig. 2. The effects of varying the temperature of the carotid body upon breathing. Initial cooling in figures A and B. Initial heating in figure C. Temperature markings (second line from bottom of records) refer to the blood at the moment of entry into the external carotid cannulae. Solid white blocks on this line indicate moments of beginning or reversal of a temperature trend. *R*, spirometer record of breathing; *AP*, arterial pressure, and *PP*, perfusion pressure recorded with mercury manometers. Time, bottom line, is in seconds and in 10 second blocks. See text for details of individual records.

ventilation produced by cold was of less than 2 minutes' duration and would scarcely be expected to have called forth as marked an exaggeration of the subsequent response to hyperthermia as that noted.

The changes in minute volume accompanying variations in carotid body temperature were accomplished by changes both in rate and in depth of

breathing. The marked individual differences in the relative degrees in which these two factors were displayed may be realized by comparing figure 2A, where the change in rate predominates, with figure 2B where

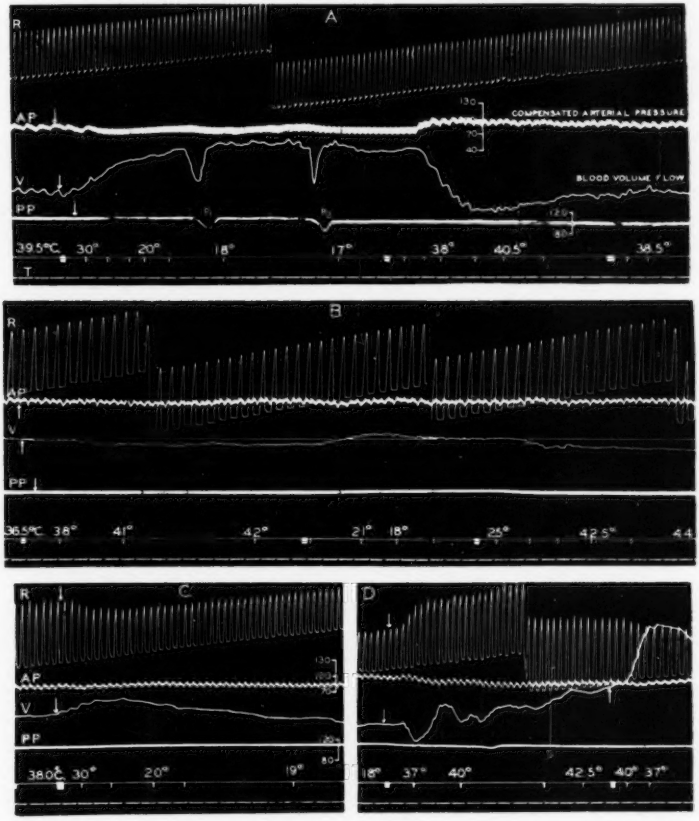

Fig. 3. Vasomotor effects of varying carotid body temperatures. Initial cooling in figures A and C. Initial heating in figures B and D. *V*, changes in blood volume flow in right axillary artery (thermoelectric method) perfused at pressure *PP*. Constant artificial pulmonary ventilation with pneumothorax in figures A and B. Natural ventilation in figures C and D. Vago-aortic nerves cut in all cases. In figure A, at P_1 and P_2, perfusion pump momentarily unable to cope with markedly increased blood flow until pump stroke increased.

rate changes are negligible and changes in amplitude predominate. More often than not changes in amplitude of breathing were more conspicuous than changes in rate. The changes appeared promptly, as nearly as can be estimated almost immediately following the arrival of the changed blood

at the carotid bodies. Surprisingly enough, they were usually well maintained over periods as long as 3 to 5 minutes (see figs. 2A and 2B) despite the altered aeration of the blood supply of the cerebral circulation and of other chemoceptive zones (the aortic bodies) not included in the perfusion circuit.

Reflex vasomotor changes (fig. 3) resulting from changes in carotid body temperature were consistent with the respiratory changes already described. Thus, cooling the carotid body caused increased blood volume flow, indicating vasodilatation and decreased vasomotor tone, while warming the carotid body caused decreased blood volume flow, indicating vasoconstriction and increased vasomotor tone. These are results which are contrary to those of Zbyszewski and others, cited earlier. Constant artificial pulmonary ventilation was used in experiments in which vasomotor changes were studied in order to avoid altered aeration of the blood supplying the vasomotor center. The necessity for this precaution is indicated by figure 3C, taken from the same experiment as 3A but just preceding the beginning of artificial ventilation. The reflex vasodilatation resulting from cooling the carotid body not only quickly disappeared, but even gave way to vasoconstriction while the cooling was still in progress, this reaction being the effect, presumably, of the poorly aerated blood acting directly upon the vasoconstrictor center. Again, in figure 3D, the initial effect of a rise in temperature at the carotid body is vasoconstriction in the foreleg, but this is quickly masked by the vasodilator influence accompanying the hyperventilation. That a vasoconstrictor influence, even though masked, had been exerted by the carotid body during the period of hyperthermia is attested by *further* vasodilatation which accompanies the reversal of hyperthermia, this dilatation occurring despite a diminishing pulmonary ventilation and an already hyperemic condition of the tissues.

Though reactions such as those shown in figure 3 are marked and clear cut, some of the experimental animals displayed vasomotor responses which were small or poorly maintained even though their respiratory reactions were definite. In two experiments the blood volume flow remained constant throughout wide variations in carotid body temperature. That the carotid body vasomotor reflex mechanism was intact in these animals was indicated by the usual marked reflex vasoconstriction upon intracarotid administration of NaCN.

Discussion. Hyperpnea and limb vasoconstriction arising reflexly from the carotid body are indications of increased chemoceptor discharge. Likewise, diminished breathing and vasodilatation are signs of decreased chemoceptor activity. The results of these experiments therefore constitute proof that increased temperature stimulates the carotid chemoceptors and that decreased temperature diminishes their activity. As we

have already suggested, these results are in harmony with the conception that increased intracellular acidity bears a causal relationship to increased chemoceptor discharge. It is proposed that increased temperature increases chemoceptor discharge by intensifying a stimulus for that discharge and that a stimulus which is intensified is intracellular hydrogen ion concentration.

Before proceeding with other phases of this discussion it may be well to inquire upon what grounds we are justified in postulating an increased intracellular hydrogen ion concentration as a result of increased temperature. There is, of course, no direct evidence that this is so. However, it is an axiom in physiology that van't Hoff's law is applicable to chemical reactions occurring *in vivo* and the effects of increased temperature in increasing the metabolic rate of tissues generally is well known. An increased rate of either aerobic or anaerobic metabolism will, as a matter of course, increase the rate of production of (acid) metabolites and hence the hydrogen ion concentration of the cell interior. In addition, there are reasons for believing that increased temperature may influence the acidity of cells in other ways. It has been shown by Stadie and Martin (1924), Cullen, Keeler and Robinson (1925) and Stadie, Austin and Robinson (1925) that a rise in temperature increases the base-binding capacity of blood proteins (in vitro), causing them to combine with more base and thus liberate more or combine with less CO_2. It is not unreasonable to suppose that similar changes may occur "in vivo" under the influence of temperature changes and that they may involve proteins within tissue cells as well as those of the blood.

While they do not provide direct evidence concerning the state of affairs in the cell interior itself, the experiments of Bazett and McGlone (1928) strongly encourage the viewpoints outlined above. These authors observed the effects of various temperatures upon the skin color of cats and men, previously injected intravenously with phenolsulphonephthalein, and concluded that skin acidity varies with skin temperature, a leg immersed in a warm bath showing a greater acidity than one immersed in a cold bath. The maximum temperature effect in their experiments was an estimated pH difference of 0.5 unit between temperature extremes of 12.5° and 40.5°C., and the smallest effect was a pH difference of 0.1 over a temperature range of 15° to 33°C.

In applying these concepts to a specific tissue, such as the carotid body, the question must arise as to whether or not there are compensating mechanisms which may counteract or prevent the development of the acid change. Increased transport of CO_2 away from the tissue due to respiratory and circulatory adjustments might play this rôle. Respiratory compensation for increased temperature would consist of increased aeration of arterial blood thereby lowering its CO_2 tension and increasing the

diffusion gradient between tissue cells and blood. The latter factor, while it is undoubtedly active under ordinary conditions, is prevented in these experiments from operating in behalf of the carotid body cells by the nature of the perfusion circuit. The perfusion pump with its connecting tubes has a relatively large capacity and the normally aerated blood which it contains at the beginning of any experimental procedure is sufficient to supply the carotid body for some minutes before the hyperventilated blood completes the circuit. Circulatory compensation for increased temperature would occur if the vessels dilated, thus increasing the facilities for transport of CO_2 from the tissue. While nothing is known concerning the control of the blood vessels of the carotid body, it is highly probable that such a compensatory phenomenon may occur. Freeman and Zeller (1937) have demonstrated the operation of a very effective and accurate local mechanism for adjusting the blood flow to the increased local metabolic rate resulting from increased temperature in the sympathectomized paw of the dog. The volume flow of blood varied directly with the temperature while the arterio-venous O_2 and CO_2 differences remained constant. It may be pointed out, however, that vascular adjustment of this type can lessen but cannot prevent the accumulation of CO_2 in the tissues when their CO_2 production is increased. For, after all, the surface area of the cells themselves must remain virtually constant and unless the CO_2 tension in the blood and tissue spaces is actually diminished, the only means available for moving extra quantities of CO_2 out of the cell is to increase the CO_2 diffusion gradient by increasing the intracellular CO_2 tension.

Another compensatory factor which deserves analysis is the effect of temperature variations upon the oxygen combining properties of hemoglobin. As Barcroft and King (1909) have shown, the dissociation curve for oxyhemoglobin is lowered by an increase of temperature. It follows that the heating of oxygenated blood contained in a closed system of tubes, as it was done in our experiments, must have resulted in a raised tension of O_2 in the plasma and so in an increased O_2 supply for the carotid body. This would tend to prevent the increased O_2 requirement of the warmed chemoceptors from outstripping their O_2 supply. The altered behavior of hemoglobin is thus a compensating mechanism in the sense that it opposes the intervention of anaerobic metabolism and the more intense production of acid metabolites which anaerobic metabolism entails. However, it would in no way prevent increased formation of the acid products of aerobic metabolism and must, therefore, be classified, with vascular adjustments, as a factor capable of lessening but not preventing a rise of intra-cellular hydrogen ion concentration of chemoceptors when they are subjected to the influence of increased temperature.

Tonic chemoreflex respiratory stimulation. That the carotid body chemo-

ceptors are moderately active (anesthetized cat) under resting conditions and at arterial oxygen and carbon dioxide tensions characteristic of such conditions has been shown by the nerve potential studies of Zotterman (1935) and of Samaan and Stella (1935). But the extent to which this moderate activity may exert a tonic stimulating effect upon respiration has not been definitely established. Selladurai and Wright (1932) reported markedly diminished breathing of the cat when the carotid bifurcations were denervated. The method of denervation was not clearly stated, but is described elsewhere (Stella, 1935) as involving the sectioning of several nerves other than Hering's. Further, the denervations were preceded earlier in the experiments by ligation of the internal carotid arteries which, in the cat, may markedly interfere with the intracranial circulation. Witt, Katz and Kohn (1934) observed respiratory failure in 34 of 45 animals following denervation of the carotid sinus regions. The drastic nature of these results and especially the fact that respiratory failure sometimes occurred following denervation of but one side strongly suggests that other factors in addition to the denervation were involved. Stella, using both anesthetized dogs (1935) and decerebrate cats (1936), often observed a persistent reduction of breathing following functional interruption of Hering's nerves alone. v. Euler and Liljestrand (1936) observed increased alveolar carbon dioxide tension following denervation of the carotid sinus region. Gesell and Lapides (1938) observed occasionally, but not consistently, that depression of eupneic breathing followed cold blocking of Hering's nerves.

While some of the experimental results cited above, especially those of Stella, undoubtedly indicate tonic respiratory stimulation by chemoceptors, yet they cannot be viewed as *purely* the effects of chemoceptor removal, for interruption of Hering's nerve entails the breaking of pressoreceptor as well as chemoceptor centripetal pathways. Quantitative evaluation of the results of chemoceptor removal are thus impossible by this technique since pressoreceptor inactivation by itself can influence breathing in several ways. Removal of the normally inhibiting effect of the pressoreceptors upon breathing would tend to oppose the effects of chemoceptor denervation. On the other hand the circulatory consequences of pressoreceptor denervation (augmented cerebral circulation because of arterial hypertension) might tend to reinforce and exaggerate the effects of chemoceptor denervation. In any case, it would be difficult to evaluate the resultant of these effects so that proper allowance could be made for them.

By our procedure these difficulties are effectively circumvented, for cold perfusion of that portion of a vascularly isolated carotid bifurcation which lies above the Gollwitzer-Meier ligature accomplishes selective inactivation of chemoceptors while leaving pressoreceptors intact and func-

tioning within normal levels. The quantitative values afforded by the present experiments thus assume added significance, since they must be viewed as purely the effect of removal of the carotid body chemoreflex influence. Reduction of breathing by as much as 34 per cent of the normal due to cooling of the carotid body is indicative of a chemoreflex tonic control of breathing of significant proportions. Further, removal of this tonic control is at least not immediately compensated by other mechanisms, as indicated by figure 2A where at the end of 4 minutes of cold perfusion the respiratory depression is still in evidence. Whether a similar degree of tonic chemoreflex activity is to be found in the unanesthetized animal is a matter for further investigation.

SUMMARY AND CONCLUSIONS

The carotid body zones of dogs, vascularly isolated from the carotid sinus pressoreceptors and all other regions, were perfused under constant hydraulic conditions with normally aerated blood at temperatures varying within the limits of 15°C. and 45°C.

Increased temperature caused reflexly increased breathing and reflex vasoconstriction, which are indications of increased chemoceptor activity.

Conversely, decreased temperature caused reflexly diminished breathing and reflex vasodilatation, which are indications of lessened chemoceptor activity.

These findings demonstrate a further correspondence between changes in chemoceptor activity and theoretically concurrent changes in chemoceptor acidity and they are therefore considered to support the hypothesis that the functional activity of chemoceptors is dependent upon the rate and quality of their own acid metabolism as well as upon the properties of their environment.

Cold perfusion as performed in these experiments constitutes selective inactivation of the chemoceptors alone. Since such chemoceptor inactivation caused reduced breathing up to 34 per cent of the normal, it is concluded that, in anesthetized animals, the carotid chemoceptors exert a tonic stimulating effect of significant proportions upon the respiratory center.

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MEASUREMENT OF THE BLOOD FLOW OF THE SPLEEN

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The rôle of the spleen as an organ of the vascular system has been investigated by a variety of methods. The thermostromuhr permits the measurement of the flow of blood in the intact splenic artery or vein of the trained animal. The recording of the volume of the spleen by inclosing it in a plethysmograph and the measurements of splenic area after surgical procedures which allow direct or roentgenographic observation have extended our knowledge of the physiology of the spleen. The spleen has been observed to dilate and fill with blood under some conditions and to contract under other conditions. The studies of splenic volume, which have provided evidence of this kind for the blood reservoir function of the spleen, are subject to the theoretic criticism that the methods employed expose the spleen to a foreign environment. The purpose of the present experiments, in which no procedures were performed on the spleen itself, was to measure inflow and outflow of blood during rest and various experimental conditions. The evidence for the blood reservoir function of the spleen from studies of the splenic volume could then be considered in connection with evidence obtained remote from the spleen itself; in addition, the flow of blood through the spleen could be studied in relation to certain well-known physiologic conditions in which contraction or dilatation of the spleen has occurred.

An early investigation of splenic blood flow was made by Burton-Opitz (1). He reported the blood flow of the splenic vein of the anesthetized dog to be 58 cc. per minute per 100 grams of spleen. Mertens (2) measured relative changes in the blood flow of the splenic vein and artery of anesthetized dogs by the thermostromuhr method. After administration of epinephrine, stimulation of one of the splanchnic nerves, of the sciatic nerve or of the splenic nerves, asphyxia, cold, or muscular work he observed an interval during which the blood flow of the splenic vein was increased and exceeded the blood flow of the splenic artery. Concerning the blood flow of the splenic artery in this brief interval he observed decreases after some procedures and slight increases after others. He found it difficult to account for this except on the basis of a relation of the arterial

flow to both blood pressure and a postulated factor of resistance in the splenic vascular network.

METHODS. Blood flow was measured in the splenic artery or vein by means of the thermostromuhr developed by Rein and modified by Baldes, Essex and one of us (3, 4) (Herrick). From repeatedly checked calibrations the deflections of a sensitive d'Arsonval galvanometer, observed directly on a scale or photographed on moving photosensitive paper, could readily be translated into blood flow in cubic centimeters of blood per minute.

Healthy dogs weighing 10.8 to 26.5 kgm., trained to lie quietly on a table and in some cases also to run on a treadmill, were fasted for a day before operation. Under ether anesthesia and with aseptic technic the splenic pedicle was exposed through a midline abdominal incision and the splenic artery or vein carefully separated from the nerves and cleaned of connective tissue for a distance of about 1.5 cm., just proximal to the point of bifurcation of the vessel. In the case of the splenic artery a few small nerve fibers were necessarily torn. Of several well calibrated thermostromuhr units sterilized by boiling for thirty minutes and ranging from 2 to 3.5 mm. in diameter, one which fitted the vessel snugly was chosen and secured in place. The vasa brevia and a few small veins to the tail of the pancreas from the splenic vein were ligated. In order to delay adhesions and the formation of collateral blood vessels to the hilum of the spleen, the gastrosplenic ligament and greater omentum were removed. The rubber insulated, flexible, lead wires were led through a stab wound in the left hypochondrium after first suitably anchoring them to peritoneum covering the left kidney capsule and abdominal wall. Several experiments, in which the blood flow of the splenic vein and splenic artery or of the splenic vein and portal vein were measured simultaneously, were performed on anesthetized dogs with the same technic except that aseptic precautions were not taken and anesthetics other than ether were often used.

The only effect of the operation and the presence of the unit and lead wires was occasionally to cause a temperature rise of 1 or 2° (F.) for one or two days, and slight serous drainage from the stab wound. The dogs continued to be healthy and active as long as the unit was present.

RESULTS. *Rest and sleep.* Barcroft and Barcroft (5) demonstrated sequestration of splenic blood from the general circulation during rest and Hanak and Harkavy (6) found that it is only the stored blood which acts as though it were outside the general circulation. Barcroft and his coworkers (7) found that carbon monoxide kills splenectomized animals sooner than intact animals. Studies of the blood in the spleen during rest have indicated that it is richer in red blood cells than blood elsewhere (8–10), and that when blood is expelled into the general circulation by

splenic contraction polycythemia results. More recently Knisely (11) has described the storage of blood cells in the sinusoids of the spleen during resting conditions.

The values for the blood flow in the splenic artery or vein of the trained dog during rest were obtained in our study after the dog had been resting quietly for a suitable period on a comfortable pad in a room free from environmental disturbances. Frequently the dogs fell asleep. The average resting blood flow was 97 cc. per minute for the splenic artery group of dogs and 91 cc. per minute for the splenic vein group of dogs. These values represent the average of the daily observations for all dogs within each group. A relationship between the size of the dog and blood flow was noted; however, exceptionally obese and phlegmatic dogs had lower blood flows when compared with other dogs on a basis of flow of blood for a unit of body weight. Seven observations during sleep revealed no change in the blood flow of the splenic artery or vein.

Noise. Hargis and one of us (12) (Mann) described contraction of the spleen in response to a sudden noise. Corbeille and Baldes (13) confirmed this observation. Others have described various degrees of contraction of the spleen in response to environmental changes in which there was an element of surprise.

We observed a 38 to 154 per cent increase of flow in the splenic vein for a minute in four experiments in which the stimulus was a loud, sudden noise. The increase was followed by a slight decrease of flow for another minute. In one experiment there was no change in the flow of the splenic vein. There was no change in the flow of the splenic artery in five experiments.

Feeding. As early as 1830 Dobson (14) observed larger spleens in recently fed dogs. Hargis and one of us (Mann) found that the spleen is enlarged from four to ten hours after feeding. Hou and Lim (15) described slight contraction of the spleen for the first fifteen minutes after feeding. Knisely (11) has described storage of blood cells in the sinusoids of the spleen after feeding.

Our study of splenic blood flow during digestion consisted in measuring the effect of feeding a standard cooked meal with a meat base to dogs which had been fasted for twenty-four hours. The flow of blood in both the splenic artery and the splenic vein increased gradually after the meal to a sustained plateau level. The magnitude of the increase was from 26 to 100 per cent and lasted from three to five hours. The flow then decreased slowly to the control level (fig. 1). The pulse rate invariably increased while the blood flow was increasing.

Shivering from decreased room temperature. Barcroft and Elliott (16) demonstrated contraction of the spleen when dogs were subjected to a room temperature of 0°C. We found that lowering of the room temperature was attended by the onset of shivering when the temperature had

decreased to 21° or 22°C., and a simultaneous increase of arterial and venous flow. The increase was maintained at a plateau level for as long as the animal shivered; the flow returned to the control level as soon as shivering ceased, when the room temperature was allowed to rise.

Exercise. That exercise causes contraction of the spleen has been shown by Barcroft (17), Barcroft and Elliott (16), and Hou and Lim (15). Barcroft and Stephens (18) found that contraction of the spleen in exercise occurs as an early event. Knisely (11) described emptying of the splenic sinusoids in exercise and the rapid passage of arterial blood through the contracted sinusoids and capillary shunts.

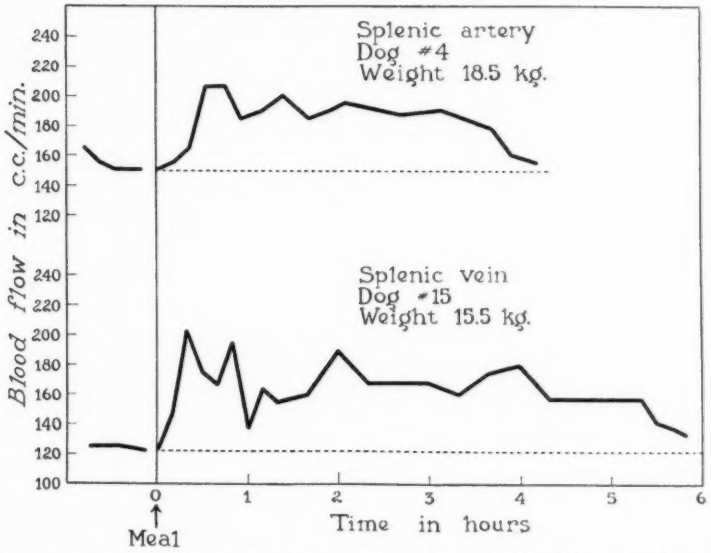

Fig. 1. (Dogs 4 and 15.) The effect of feeding on the blood flow in the splenic artery (above) and splenic vein (below).

Our experiments on the effect of exercise were performed on a treadmill which was operating at a speed of 3 miles an hour. We observed a maintained increase of blood flow in both the splenic artery and the splenic vein during exercise (table 1). In the case of the splenic artery there was little immediate change of flow, usually a slight increase or decrease, but gradually the blood flow increased to a plateau level. The flow in the splenic vein showed a sharp increase lasting approximately one minute, followed by a decline of the blood flow to a level nearly always above the control flow. Following the cessation of exercise the control level of arterial or venous flow was reached in zero to twenty-one minutes; the arterial

flow always decreased gradually while the venous flow always decreased sharply during the first minute after the cessation of exercise, then increased again and decreased gradually.

Hemorrhage. The early observations of Dobson (14), Gray (19) and Crisp (20) that the spleen is shrunken after hemorrhage have been confirmed by Barcroft (17), and Hou and Lim. Barcroft and his co-workers (21) showed that the spleen gives up the bulk of its blood at the beginning of hemorrhage.

We observed a marked and maintained decrease in the splenic blood flow in hemorrhage. During an arterial hemorrhage of 10.6 per cent of the

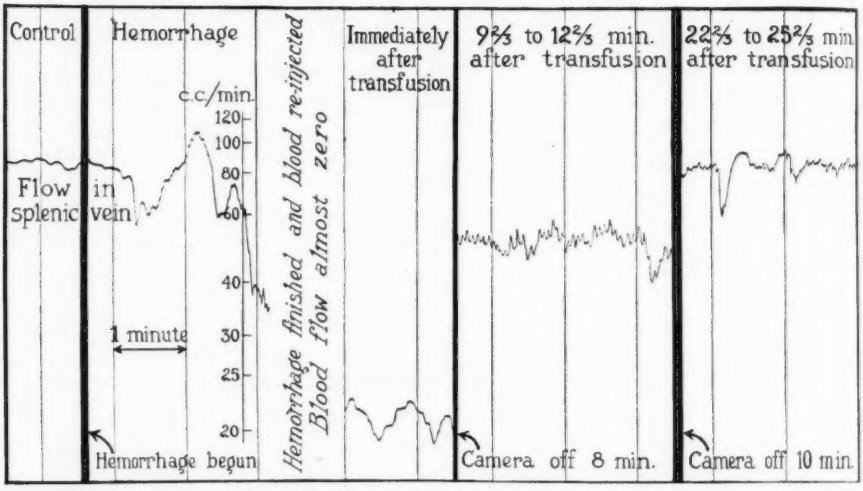

Fig. 2. (Dog 16.) The effect of sudden arterial hemorrhage of 30 per cent of the estimated total blood volume, and replacement of the same blood with added heparin, on the blood flow in the splenic vein.

estimated total blood volume in $2\frac{1}{2}$ minutes the blood flow in the splenic artery in one dog decreased from the control level of 59 cc. per minute to 27 cc. per minute (54 per cent decrease) at the end of hemorrhage; the decrease was maintained. In another dog (fig. 2) an arterial hemorrhage of 30 per cent of the estimated total blood volume in $3\frac{1}{4}$ minutes caused the flow in the splenic vein to decrease from 78 cc. per minute to almost zero by the end of hemorrhage. The decrease in blood flow in the vein during hemorrhage was marked by a preliminary, transitory period of increased blood flow. The same blood, containing added heparin (nontoxic solution, Connaught Laboratories) was rapidly reinjected and the blood flow reached the control level in $22\frac{2}{3}$ minutes after transfusion. The experiment was repeated on this dog with the same results.

Intravenous infusions. Barcroft (22) observed dilatation of the spleen after relatively large quantities of normal saline solution had been suddenly thrown into the circulation. Hargis and one of us (Mann) (unpublished work) found confirmatory evidence for this.

We nearly always observed (table 2) increases in the blood flow of the splenic vein or splenic artery during the intravenous injection of 150 cc. to 350 cc. of Ringer's solution, 10 per cent glucose in Ringer's solution, 20 per cent sucrose or whole, heparinized blood. The blood flow in both the splenic artery and the splenic vein increased gradually to the maximum flow at the end of the infusion. The flow in some cases then decreased rapidly and in other cases, usually when the dog was restless, decreased slowly. The smallest increases of flow were produced by Ringer's solution, particularly when the blood flow was already relatively high.

Anesthesia. During ether anesthesia contraction of the spleen has been observed by Hou and Lim (15), and by Hausner, Essex and one of us (23) (Mann). The latter authors reported dilatation of the spleen during barbiturate anesthesia. We compared the effect of ether and sodium pentobarbital anesthesia on splenic blood flow. In one experiment ether anesthesia caused no sustained change in the blood flow of the splenic vein and in one experiment a 17 per cent decrease. We were unable to observe the flow in these experiments during the induction of ether anesthesia. In four experiments, in two of which the arterial flow was observed and in two the venous flow, sodium pentobarbital anesthesia produced an increase of blood flow. The increase began during the induction state and gradually reached a plateau level of increase of 25 to 44 per cent (average 32.6 per cent).

Drugs. Various pharmacologic preparations of internal secretions and drugs acting on the vasomotor system have been extensively studied by many investigators (12, 15, 22, 24–29) for their action on the spleen. Epinephrine, ephedrine, pituitrin, histamine, acetylcholine and nitrites were shown to cause various degrees of contraction of the spleen. Knisely (11) described the emptying of the splenic sinusoids after stimulation with epinephrine. Mertens (2) found a preliminary increase of flow in the splenic vein and a simultaneous briefer and lesser increase of arterial flow; the increases in both venous and arterial flow were succeeded by sudden decreases below the level of control flow, with gradual recovery to the control flow.

In this study, doses of epinephrine, ephedrine, pitressin, histamine, acetylcholine and glyceryl trinitrate, diluted with 1 to 2 cc. of Ringer's solution, were injected into the saphenous vein and the effect on the blood flow of the splenic artery or vein observed. No significant effect was produced by the introduction of the needle or when equivalent quantities of Ringer's solution alone were injected.

Epinephrine (0.005 cc. 1–1,000 per kgm. of body weight) was used in six experiments in which blood flow was observed in the splenic artery and five in which flow was observed in the splenic vein. The blood flow in both vessels underwent a marked, transitory increase, an average of 208 per cent in 50 seconds in the splenic artery group and 217 per cent in 39 seconds in the vein group. The increase was succeeded by a period of decreased blood flow, an average decrease below the control level of 44 per cent at 3 minutes in the splenic artery group and 43 per cent at $3\frac{2}{3}$ minutes in the vein group. Recovery to the control level of blood flow was gradual and occurred at an average of 8 minutes after injection in the artery group and $6\frac{1}{2}$ minutes in the vein group. In both the splenic artery and the splenic vein groups there was one experiment which differed from others in that group in that the blood flow ultimately returned to the control flow by way of a gradual decrease from the peak of increased flow, not by way of a gradual increase from a period of decreased flow.

Ephedrine (1.2 mgm. ephedrine hydrochloride per kgm. of body weight) was used in one experiment in which blood flow in the splenic artery was measured and three experiments in which the venous flow was measured. The effect, which was similar to that caused by epinephrine, consisted of a small transitory increase that was greater in the case of the venous flow than of the arterial flow. The increase was followed by a profound and prolonged decrease of flow and a slow return to the control flow at an average of 15 minutes.

Pitressin (0.05 cc.) was used in two experiments in which splenic arterial flow was measured and one experiment in which venous flow was measured. Both the arterial and the venous flow were markedly decreased to a level from which recovery was not complete for 21 to 33 minutes. The arterial flow decreased suddenly; the decrease in venous flow occurred later and was more gradual.

Histamine (1.0 mgm.) was used in three experiments each in which splenic arterial or venous flow was measured. The arterial flow decreased markedly and suddenly, and returned to the control level slowly. The venous flow likewise diminished, but the decrease was preceded by a brief period of increased blood flow.

Acetylcholine (1.0 mgm.) caused only a slight brief decrease of the arterial flow of the spleen in one experiment. In two experiments, in which the dogs had been given 1 mgm. of eserine 20 minutes previously, smaller doses of acetylcholine caused a brief rise of the flow in the splenic vein; when 0.1 mgm. of acetylcholine was used, the flow increased briefly and gradually diminished to the control rate of flow; when 0.5 mgm. was used the brief preliminary increase was followed by a decrease to a sub-control level of flow before the flow finally stabilized at the control level.

Glyceryl trinitrate (2.2 mgm.) was used in four experiments in which

splenic arterial flow was measured and three experiments in which venous flow was measured. The mean arterial blood flow did not change; the venous flow underwent a moderate, brief increase after the administration of glyceryl trinitrate.

Simultaneous measurement of blood flow in the splenic vein and the splenic artery. The blood flows of the splenic artery and splenic vein were measured simultaneously in five experiments on anesthetized dogs. These simultaneous observations, recorded photographically, established the fact that the blood flows in the two vessels were usually almost exactly the same. Disparities in arterial and venous flow, when found, showed a tendency to disappear when the observation was continued for a period of several minutes. In one experiment an attempt was made to learn whether consistent variations of arterial and venous flow were related to variations in the size of the spleen, as recorded by a sensitive plethysmograph (1 cc. change in volume equivalent to 6 cm. deflection on photographic record). The simultaneous arterial and venous flow recordings, read at one minute intervals, showed a progressively decreasing arterial flow and increasing venous flow. A photographic recording of changes of splenic volume during this interval furnished the evidence that the spleen was enlarging at the one minute interval, when the inflow exceeded outflow; that no change was occurring at three to four minutes, when inflow and outflow were equal; and that thereafter, when outflow exceeded inflow, there was a slight shrinkage of the spleen.

In these experiments on anesthetized dogs the effect of epinephrine on splenic blood flow, as measured in the artery and vein simultaneously (fig. 3), resembled the effect of the drug on trained dogs, as measured in the splenic artery or vein in different dogs. It was evident, however, that the changes of arterial flow were of lesser magnitude than the simultaneous changes of venous flow in the anesthetized dogs; in the trained dog observations on the artery and vein in different dogs had shown no consistent differences in the average magnitude of changes in the two flows.

Simultaneous measurement of blood flow in the splenic vein and the portal vein. The blood flow of the splenic vein and portal vein was measured simultaneously in three experiments. In two experiments under pentobarbital sodium anesthesia the flow in the splenic vein was an average of 10.7 and 35.5 per cent of the flow in the portal vein. In one experiment under ether anesthesia the flow in the splenic vein was an average of 5.23 per cent of the portal flow. In addition to these simultaneous recordings of flow in the splenic and portal veins the portal flow was obtained in another experiment before and after splenectomy; in this experiment, under pentobarbital sodium anesthesia, the decrease in portal flow after splenectomy amounted to 27 per cent.

Evidence was obtained in two experiments (table 3) that at the time the

TABLE 1

The effect of exercise on splenic blood flow

NUMBER OF DOG	SPLENIC VESSEL	MEAN CONTROL FLOW	DURATION OF EXERCISE	CHANGES UP TO 1 MINUTE	MAINTAINED LEVEL OF INCREASE
		cc./min.	*min.*		*per cent*
5	Artery	50	9	Slight decrease	106
		55	11	Slight increase	191
3	Artery	135	22	Slight decrease	37
		192	5½	Slight decrease	19
8	Artery	88	8	Slight increase	40
		173	11	Slight increase	16
		245	10	Slight increase	10
12	Vein	43	7	67 per cent increase	40
		11	7	100 per cent increase	0
13	Vein	162	6	20 per cent increase	15

TABLE 2

The effect of intravenous infusions on splenic blood flow

SUBSTANCE INJECTED	NUMBER OF DOG	SPLENIC VESSEL	FLUID INJECTED	CONTROL BLOOD FLOW	MAXIMUM PERCENTAGE INCREASE IN FLOW
			cc./kgm. body wt.	*cc./min.*	
Ringer's solution	7	Artery	11.6	70	0
	7	Artery	11.6	51	20
	11	Artery	25	39.5	44
	15	Vein	13	34	56
10 per cent glucose in Ringer's solution	4	Artery	16.2	161	130
	6	Artery	17.7	78	56
	14	Vein	11.8	58	83
20 per cent sucrose	11	Artery	16.2	99	102
	16	Vein	17.7	68	54
	20	Vein	11.8	4	500*
Heparinized blood	19	Vein	15.4	109	62
	11	Artery	16.9	54	18
	16	Vein	12.9	20	18
	20	Vein	13.9	1.5	1100†

* The blood flow actually increased to 24 cc. per minute.

† The blood flow actually increased to 19 cc. per minute.

flow in the splenic vein increased after the injection of epinephrine, the flow in the portal vein decreased markedly. The experiment on portal flow before and after splenectomy, accordingly, was undertaken to learn why the portal flow should decrease at the moment after the injection of

epinephrine that the flow of the tributary splenic vein increased. It was learned that before splenectomy the usual decrease in portal flow occurred following the administration of epinephrine, but after splenectomy a much greater decrease occurred (table 3).

COMMENT. Aside from technical difficulties the chief sources of error in the thermostromuhr method of measuring blood flow lie in 1, turbulent flow, and in 2, slight quantitative underestimation of changes of flow occurring in less than one minute. These errors were recognized; when turbulence was suspected, experiments were discarded. In cases where the increase or decrease lasted less than one minute the recorded change undoubtedly was of lesser magnitude than if the change had occurred over a greater period of time. The change in flow during the first minute after a noise, exercise or administration of epinephrine should be regarded as conservative estimates of the true quantitative increases or decreases. Evidence that normally the blood flow in the splenic artery and vein is streamline and not turbulent was obtained from the experiments on anes-

TABLE 3

Effect of epinephrine on splenic vein and portal vein flow; flow in cc./min.

NUMBER OF DOG	VESSEL	CONTROL	½ MINUTE	1½ MINUTE
27	S.V.	24	217	17
	P.V.	242	152	360
36	S.V.	121	195	140
	P.V.	309	230	600
37	P.V. before splenectomy	310	300	600
	P.V. after splenectomy	225	29	400

thetized dogs, in which the almost identical arterial and venous flows made it unlikely that the flow was turbulent in either vessel.

From the results of our study it would seem desirable, in the case of an organ so variable in size as the spleen, not to follow the example of Burton-Opitz (1), who expressed flow in terms of organ weight.

The finding, from splenic volume and other methods of investigation, that blood stored in the spleen is ejected during splenic contraction has been confirmed in this study by the fact that venous flow exceeded arterial flow for an interval after contraction-producing stimuli. The additional data which a study of the blood flow of the spleen furnishes concern findings which have not been anticipated by other methods. When contraction of the spleen has occurred the commonly held opinion is that the blood flow is depressed. This is true after contraction produced by hemorrhage or certain drugs. It is not true for the splenic contractions produced by exercise, cold, ether anesthesia, noise, and occasionally epinephrine.

After all of these stimuli, contraction of the spleen has been described and in this study was in most cases confirmed by a temporarily greater venous than arterial flow. However, in exercise and during cold this interval was followed by a state in which arterial and venous flow were greater than the control flow; after a noise the interval was followed by the control rate of flow; during ether anesthesia there was little or no sustained change of flow; and in one experiment each the splenic artery and vein failed to undergo the usual period of decrease of flow after the administration of epinephrine. From these results it is clear that the behavior of blood flow cannot be interpolated from plethysmographic data in the case of the spleen. The volume and variations in the volume of the spleen yield evidence of the blood stored in the spleen. The thermostromuhr indicates storage of blood or loss of stored blood by the spleen and, in addition, records circulatory changes occurring at the same time.

The circulatory changes of splenic flow which are independent of transitory reservoir changes must be related to changes within the general vascular system, of which the spleen is manifestly a part. Considered in this light, the data appear to conform to what is known of general vasomotor behavior during the same conditions. For example, in hemorrhage the decreased splenic flow is consistent with the factors of splanchnic constriction and decreased cardiac output; and in cold, exercise, intravenous infusions, and digestion the increased splenic flow is consistent with the factor of an increased cardiac output. After a noise and occasionally after the administration of epinephrine the failure of a decrease of splenic flow to occur probably indicates a balance of such antagonistic factors as increased cardiac output and resistance in the splenic vascular bed.

The work of Knisely (11) on the splenic sinusoids during rest and after feeding, exercise and the administration of epinephrine illuminates the concept that the spleen acts as a storehouse in which blood cells are stored or discharged as the circulatory system requires, and as an organ through which blood flow is regulated by the vasomotor system according to general circulatory requirements.

SUMMARY OF RESULTS. During rest the average blood flow in the splenic artery in a group of trained dogs was 97 cc. per minute, and 91 cc. per minute in the splenic vein in a similar group of trained dogs. The blood flow was the same during sleep as during rest.

A noise caused a brief transitory rise of blood flow in the splenic vein. During digestion the arterial and venous flow of the spleen underwent a sustained increase for three to five hours. Shivering from decreased room temperature caused an increase of splenic blood flow. Exercise caused both a transitory increase in splenic vein flow and a gradual, maintained increase in arterial and venous flow.

Hemorrhage caused the arterial flow to decrease rapidly and the venous flow to decrease after a transitory rise. The flow remained decreased until

after transfusion. Massive increases of blood volume resulting from the injection of isotonic or hypertonic fluids or of blood caused prolonged increases in arterial and venous flow, relatively greatest increases occurring in cases of low splenic flow.

Ether anesthesia caused the splenic vein flow to decrease slightly or not at all while pentobarbital sodium anesthesia produced a prolonged

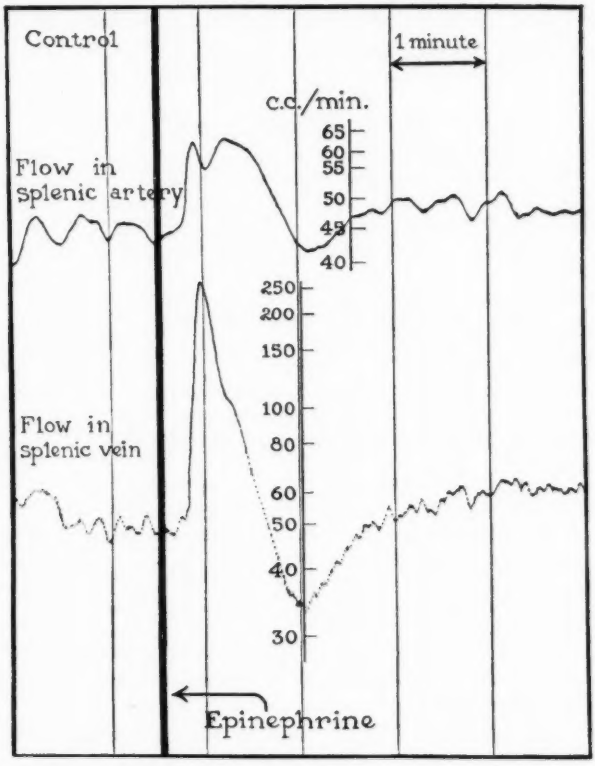

Fig. 3. (Dog 26.) Sodium pentobarbital anesthesia. The effect of epinephrine on blood flow in the splenic artery and vein.

increase of splenic flow. Epinephrine usually produced a temporary decrease of splenic arterial and venous flow, preceded by a transitory rise. Ephedrine, pitressin, histamine, acetylcholine and glyceryl trinitrate produced immediate changes in splenic flow.

In simultaneous observations on anesthetized dogs the splenic arterial and venous flows were the same, except when the splenic volume was changing.

The splenic vein contributed 10 to 35 per cent of the flow of blood in the

portal vein in dogs anesthetized with sodium pentobarbital. A rise in splenic vein flow appeared to moderate severe fall in portal flow after the injection of epinephrine.

CONCLUSIONS

The spleen appears to behave as an integral part of the vascular system: 1, blood is stored or discharged according to circulatory demand, and 2, at the same time circulating blood is allowed to pass through the spleen at a rate determined by circulatory activity.

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RHYTHMICITY OF THE SPLEEN IN RELATION TO BLOOD FLOW

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Observations of the blood flow in the splenic artery and vein in trained dogs (1) disclosed the fact that frequently the flow is rhythmic. Although the mean flow remained constant or changed in a consistent fashion there were often cyclic increases and decreases of flow. When the blood flow was recorded continuously by photography, rhythmic waves, having a definite period, were observed on the photographic record. In the trained dog the observations were made on the splenic artery and vein separately. In order to determine the relationship of arterial flow waves to venous flow waves and of blood flow waves to the well-known volume waves of the spleen, arterial and venous blood flow and splenic volume were photographically recorded simultaneously in dogs anesthetized with chloralose.

The waves obtained by a continuous tracing of the volume of a spleen inclosed in a plethysmograph, commonly called the "splenic rhythm," were first reported by Roy (2) in 1881. This finding has been confirmed by Schäfer and Moore (3), Hargis and Mann (4), and Barcroft and his co-workers (5–7). All of these investigators worked with the spleen of the dog or cat. The rhythmic waves were described as having an average period of forty-five to sixty seconds. The accepted interpretation of the splenic rhythm was that it is a manifestation of rhythmic contraction and relaxation of the splenic muscle. Loewe and Faure (8) demonstrated that isolated strips of splenic capsule exhibit no rhythmic activity.

Various stimuli that caused the spleen to contract were found to increase rhythmic activity during reëxpansion of the spleen. Splanchnic or vagal section, and denervation or even autotransplantation were shown not to abolish the splenic rhythm. Roy (2), and Hargis and Mann (4) found no correlation between splenic rhythm and fluctuations of blood pressure. However, Barcroft and Nisimaru (6, 9) found such a correlation and concluded that the splenic rhythm is usually caused or enhanced by a rapid rise in blood pressure. Moreover, they expressed the opinion that the splenic rhythm in turn causes the synchronous fluctuations in blood pressure.

Mertens (10), in the course of a study of splenic flow by the thermostromuhr method, found that during refilling of the spleen after contraction there were blood flow waves having a period of 30 to 50 seconds. Venous flow waves lagged about ten seconds behind arterial flow waves. Because the venous and arterial waves were nearly simultaneous he concluded that the rhythmic blood flow is not caused by rhythmic contractions of the spleen, and because venous flow waves lagged slightly behind arterial waves he suggested that rhythmic flow arises from rhythmic contraction of the end arteries of the spleen.

TABLE 1

Period of splenic blood flow waves in trained dogs during rest

DOG	VESSEL	RANGE OF PERIOD OF WAVES	AVERAGE PERIOD OF WAVES
		seconds	*seconds*
7	Splenic artery	38–60	47.5
8	Splenic artery	43–60	54
11	Splenic artery	31–42	36
12	Splenic artery	30–40	35
21	Splenic artery	45–45	45
13	Splenic vein	30–55	48
14	Splenic vein	30–56	41
15	Splenic vein	38–50	42
16	Splenic vein	40–50	45
17	Splenic vein	30–55	41
18	Splenic vein	50–60	55
19	Splenic vein	36–60	52
20	Splenic vein	30–60	44

Average period for all observations on all dogs = 45 seconds.

METHODS. In the series of experiments on trained dogs thermostromuhr units of suitable size were placed on either the splenic artery or the splenic vein surgically under ether anesthesia and with aseptic technic. Observations were made on succeeding days. Blood flow was recorded continuously by photographing the light beam from the recording galvanometer on moving photosensitive paper.

The experiments on dogs anesthetized with chloralose[1] were not carried out with aseptic technic. Thermostromuhr units were placed on both the splenic artery and the splenic vein in the usual fashion and the spleen was inclosed in a collodion-gauze plethysmograph. Volume changes of the spleen, detected by the plethysmograph, were recorded by means of a

[1] Chemically pure chloralose ("Chloralosane") was used. One hundred milligrams of chloralose in 10 cc. of physiologic saline solution per kilogram of body weight usually sufficed for anesthesia. The solution was boiled in order to dissolve the chloralose and cooled to body temperature just before injection.

tambour with an eccentrically attached focusing mirror. Light beams representing splenic arterial and venous blood flow and splenic volume were simultaneously recorded on moving photosensitive paper. The mean carotid blood pressure was recorded in several experiments on the same paper by means of a cannula connected by fluid-filled tubing to a tambour smaller than that used for the splenic volume.

RESULTS OF EXPERIMENTS. Blood flow waves were frequently noted in photographic recordings of splenic arterial or venous flow in resting, trained dogs; in two dogs waves were never observed; in the other dogs quiet and muscular relaxation was usually attended by rhythmic splenic flow. The waves during rest were of nearly the same period for all observations on any one day but did not compare so closely with observations on other days. The average period of the waves was 45 seconds (table 1); waves

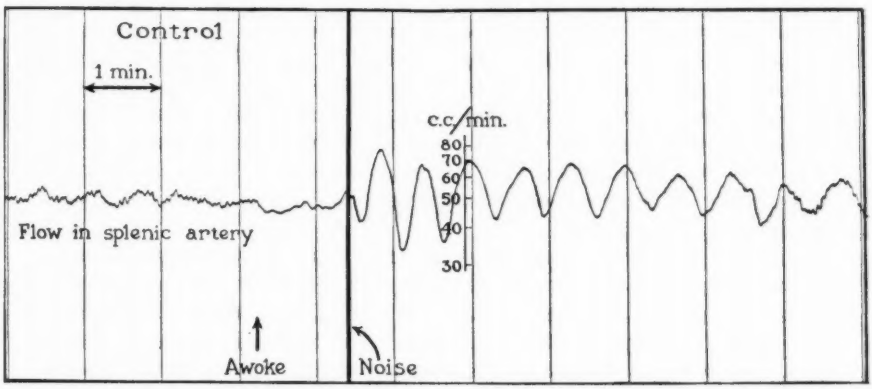

Fig. 1. (Dog 7.) Rhythmic waves of splenic arterial flow during sleep and after a sudden noise.

having a period less than 30 or greater than 60 seconds were not observed. During sleep the rhythmic waves of flow became more marked but the period of the waves decreased an average of 7 seconds. After a noise (fig. 1) the same decrease of period was noted but the amplitude of the waves, particularly in the splenic artery, was even more markedly increased. The maximal effect was observed immediately after the noise and the effect subsided in 2 to 10 minutes.

Exercise was performed by dogs trained to run on a treadmill. When blood flow waves were present during the control period they decreased both in amplitude and in period immediately after the beginning of exercise; 3 minutes later waves were no longer discernible. As soon as the treadmill was stopped blood flow waves nearly always appeared and were of rather large amplitude.

Blood flow waves disappeared during hemorrhage. In one experiment the waves did not reappear in the arterial flow for about 20 minutes. In two experiments waves reappeared in the venous flow as soon as the removed blood was replaced; these waves were of greater amplitude and of slightly shorter period. The slow intravenous injection of 150 to 350 cc. of Ringer's solution, of 10 per cent glucose in Ringer's solution, of 20 per cent sucrose or of heparinized blood did not change rhythmic flow of blood in the splenic artery or vein.

The transitory increase of arterial and venous flow in the spleen produced by epinephrine was usually followed by an interval of small blood flow. As recovery to the control level of flow occurred, blood flow waves almost invariably appeared in arterial flow and generally in venous flow. However, waves were never observed after the injection of epinephrine until the flow began to return to the control level. At the beginning of the recovery phase the waves were of large amplitude and 30 to 40 seconds in period. As the flow gradually approached the control level the waves became less prominent and longer in period. Recovery from the depressed level of flow was very slow following the administration of ephedrine, pitressin or histamine and was less often accompanied by rhythmic waves. The brief changes of blood flow produced by acetylcholine and glyceryl trinitrate were accompanied by alterations of rhythm similar to those produced by epinephrine.

In the experiments undertaken on anesthetized dogs waves were seldom observed until the beginning of reëxpansion of the spleen after an induced massive contraction. Epinephrine and rebreathing were the stimuli used to bring about splenic contraction. The rhythmic waves of blood flow, usually 30 to 40 seconds long, accompanying refilling of the spleen were similar to the waves of short period found after the injection of epinephrine in trained dogs. Rhythmic waves of arterial and venous flow and of splenic volume (the "splenic rhythm") were found to have the same period at any moment. Changes in period and amplitude were parallel.

Not only were arterial and venous blood flow and volume waves found to be of the same period but they were found to be almost exactly in phase (fig. 2). Close inspection revealed that the venous flow waves usually were not quite synchronous with the other rhythmic changes but followed them by about 5 seconds.

Denervation of the spleen, accomplished by section of the splenic nerves and all structures in the splenic pedicle except the carefully cleaned artery and vein, altered the normal response of the spleen to a stimulus consisting of $1\frac{1}{2}$ to 3 minutes of rebreathing. However, although dilatation rather than contraction took place, the usual rhythmic waves were recorded after both rebreathing and epinephrine stimulation.

Simultaneous recording of the blood pressure revealed the fact that two to four rhythmic waves of the mean pressure occurred when the spleen began to refill (fig. 3). These waves resembled and were synchronous with the splenic rhythm. Unlike the splenic waves, however, the blood pressure waves lasted only a few cycles and then abruptly changed to waves of much shorter period. No certain relationship was observed between the short cyclic variations of blood pressure and the splenic waves.

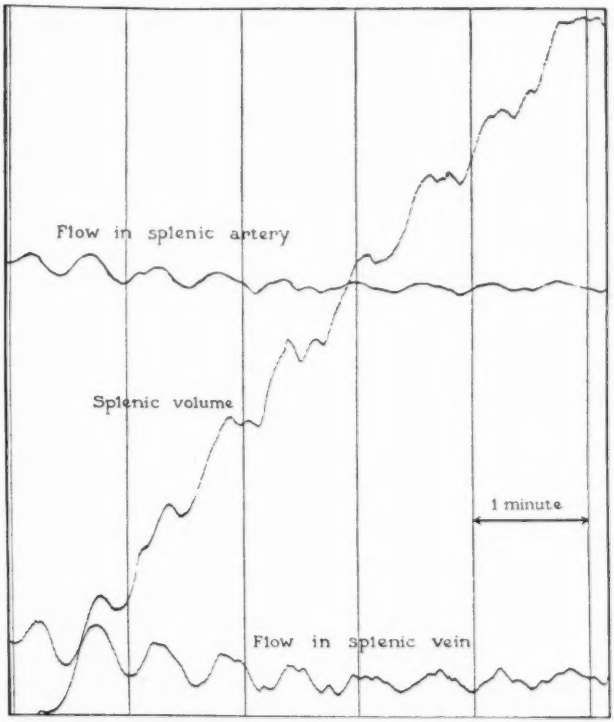

Fig. 2. (Dog 31.) Chloralose anesthesia. Rhythmic waves of splenic arterial and venous flow and splenic volume during refilling after a contraction produced by epinephrine.

COMMENT. The thermostromuhr will indicate changes of flow occurring in less than 5 seconds. This has been determined both by sudden or gradual alteration of the rate of blood flow from a perfusion pump and by plethysmographic confirmation of changes of flow in the splenic circulation.

We have confirmed the observations of Mertens (10) that the blood flow in the splenic artery and vein of the anesthetized dog may at times be rhythmic and that there is a slight lag (about 10 seconds in Mertens'

experiments and five seconds in ours (fig. 3)) of venous flow waves behind arterial flow waves.

In addition we have observed similar rhythmic waves of flow in the splenic artery or vein of the trained dog. We have found that the average period of the waves in the resting dog was 45 seconds (range 30 to 60 sec.) and that the period was somewhat shorter (up to 30 sec.) during refilling of the spleen in both trained and anesthetized dogs. We have

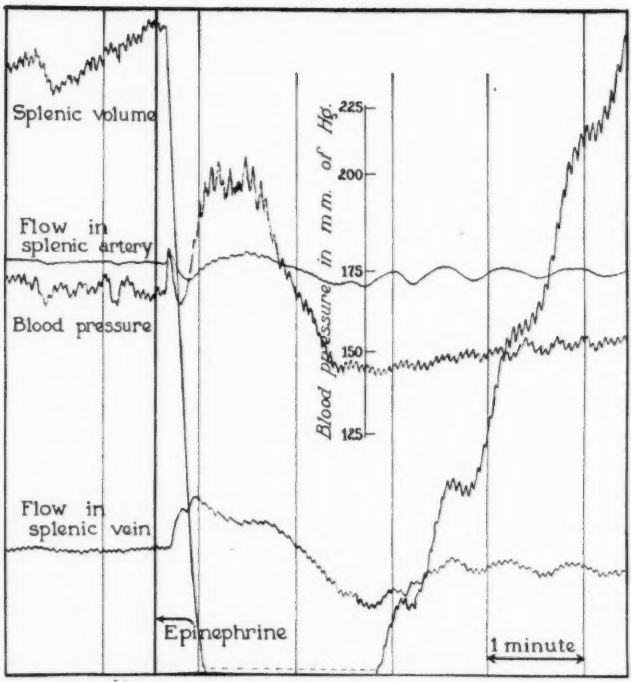

Fig. 3. (Dog 34.) Chloralose anesthesia. The relation of rhythmic waves of splenic arterial and venous flow and splenic volume to blood pressure waves after the administration of epinephrine.

observed that rhythmic changes in splenic volume, known as the splenic rhythm, resemble and are in phase with rhythmic changes of flow in the splenic artery. In the light of these facts we agree with Mertens and believe that the splenic rhythm, as Mertens postulated from experiments in which he did not observe the splenic volume, is in reality a phenomenon not of rhythmic contraction of splenic muscle but of rhythmic arterial blood flow. If the former were true the venous flow wave should be in-

creasing and the volume wave decreasing during the contraction phase. If the rhythm is due to rhythmic arterial flow, the relationships we have observed must maintain. That is, the arterial flow wave and passive wave of splenic dilatation must be nearly synchronous, and the wave of venous flow less prominent and a little later than the arterial flow wave.

From theoretic considerations the splenic rhythm may be correlated with cyclic blood flow in the splenic artery and vein in the following manner. Splenic rhythm cannot be due to contraction and relaxation of the splenic musculature unless the volume rhythm of the spleen is out of phase with venous flow rhythm. This is obvious since a contraction of the spleen itself would occur simultaneously with reduction of volume and increase of venous output. This has not been verified by our experimental data. On the other hand, if the rhythmic blood flow in the splenic artery is in phase with and equal to the venous flow there can be no splenic rhythm since the inflow to the spleen is equal to the outflow, the volume thus remaining unchanged. However, if the venous flow rhythm lags behind the arterial flow rhythm there will be in evidence cyclic changes in splenic volume, changes of maximum amplitude if the lag is 180 degrees. In our experiments the records show the average lag to be about 5 seconds, or 40 degrees.

Support of the vascular origin of the splenic rhythm is lent by experiments on denervation of the spleen. This study and previous investigations have shown that denervation does not abolish the splenic rhythm. It has been shown that denervation abolishes splenic muscular tonus, as evidenced by dilatation of the spleen, and the ability of the spleen to contract except through the agency of circulating hormones. These effects of denervation being true, it is unlikely that a regularly recurring phenomenon of contraction and relaxation would still reside as a function of denervated musculature.

In the critical experiments a few synchronous blood pressure and splenic rhythm waves were observed during rapid refilling of the spleen after splenic contraction. One suspects that the physiologic factors governing rhythmic blood flow of the spleen and possibly other organs at this time may be sufficiently active to have a secondary systemic effect that is reflected in the mean blood pressure. The presence of the splenic rhythm under other circumstances, totally divorced from blood pressure variations, is evidence that rhythmic changes in splenic blood flow are not secondary to blood pressure changes.

The absence of the splenic rhythm in circumstances where the spleen is contracted and its prominence during filling of the spleen suggests that splenic rhythm may be directly or indirectly concerned in the spleen's function of blood storage. The mass changes in blood flow during cycles of the splenic rhythm may in some manner be connected with the rhythmic

flow to blood storing and concentrating venous sinusoids described by Knisely (11).

SUMMARY. Rhythmic waves of blood flow having an average period of 45 seconds have been observed in the splenic artery and vein of the trained dog. During sleep and especially after a noise the waves both increased in amplitude and decreased in period. The same alterations in the character of the waves occurred in refilling of the spleen after contraction. During contraction and while the spleen was contracted there were no rhythmic waves of blood flow. In anesthetized dogs waves of arterial flow and splenic volume corresponded in period and were synchronous; waves of venous flow corresponded in period but lagged slightly, about 5 seconds, behind waves of volume and arterial flow. Denervation of the spleen did not abolish or disturb either the blood flow or splenic volume manifestations of the splenic rhythm. Rhythmic waves of blood pressure synchronous with waves of splenic rhythm were present during refilling of the spleen for only a few cycles of the waves of splenic rhythm.

CONCLUSIONS

The splenic rhythm of the dog is caused not by rhythmic contraction of the splenic musculature but by rhythmic blood flow in the splenic circulation. The evidence points to an arterial or arteriolar origin of the rhythmic waves of blood flow.

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AUGMENTATION OF ACTIVITY IN THE SLOTH BY ADRENAL EXTRACT, EMOTION AND OTHER CONDITIONS

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The sloth is an excellent subject for testing the excitatory effect of certain substances or conditions. Its basal level of activity is very low and increments can be readily observed. In a short while one may train the animal to travel a horizontal pole, and this it will do under ordinary laboratory conditions repeatedly and at a fairly uniform rate. The sloth is also fairly easily handled, and in the American tropics a plentiful supply may be secured.

In the course of other work observations were made on activity in two species of sloths, the two-toed *Choloepus hoffmanni* and the three-toed *Bradypus griseus*.[2] Cortico-adrenal extract, adrenalin, prostigmin and other substances were tested for their action, and the effects of emotional excitement and increases in body temperature were also noted. The tests followed a chance observation that sloths moving into the sunshine become very active as they warm up. When kept in the sun for two to four hours, however, they develop convulsive seizures and die.

A sapling tree about 15 feet long and 4 inches in diameter, suspended horizontally, served as a testing "course." At one end of this the sloth was placed in its normal upside-down position, and usually it set out immediately toward the other end. There the animal was turned face-about by an attendant, and it then retraced its steps at the same steady gait. From 8 to 12 normal "runs," constituting a series, were thus made before each experiment. Timing was done by stop-watch over a marked 12-foot length of the course.

Emotional excitement was readily set up in the sloth, as noted in an earlier paper (Britton and Kline, 1939) by making sham thrusts and sallies by hand or a whisk broom toward the animal, placed on its back in the grass, for three minutes. Exposure to the sun was made for 1 to 2

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[2] These studies were carried out mainly at the Barro Colorado Island Laboratory, C. Z., and the Gorgas Memorial Laboratory, Rep. Panama. Thanks of the authors are gratefully extended to Mr. James Zetek and Dr. Herbert C. Clark of these laboratories respectively for their unfailing help and courtesies.

hours; this usually raised the body temperature 3° to 5° above normal. Cortico-adrenal extract was found most effective in doses of 5 to 10 cc. every 2 or 3 hours; adrenalin and prostigmin were used in suitable small doses, sometimes repeated. From two to four series of tests of activity were carried out at intervals after treatment.

The results were almost invariably well defined. Typical and illustrative of the experiments are the following protocols:

March 1, 1938. Barro Colorado Island Laboratory, C.Z.
Two-toed sloth, no. 5, normal.
Excitement test. 10 a.m., walking on pole, 12 ft. course; running time, seconds per 12 ft. run, 24, 18, 18, 19, 20, 18, 21, 24, 24, 22; average, 20.8. Put animal on ground, excited by handling, etc., for 3 min. Time after excitement, seconds 16, 16, 13, 14, 13, 12, 10, 10, 14, 14; average 13.2.
Increased rate after excitement, 38 per cent.

March 4, 1938. Barro Colorado Island Laboratory, C.Z.
Two-toed sloth, no. 13, normal.
Temperature test. 12 m., rectal temp. 34.0°; walking on pole 12 ft. course; time, seconds per 12 ft. run, 28, 26, 28, 32, 30, 26, 28, 30, 30; average 28.7. Warmed in sun in cage, 1 hour; rectal temp. at 1:30 p.m., 36.1°; running times, seconds, 20, 18, 18, 17, 18, 17, 18, 19, 20, 18; average 18.3. Later runs averaged 20 sec. per 12 feet.
Increased in running rate after warming, 37 per cent.

March 28, 1939. Gorgas Memorial Laboratory, Rep. Pan.
Three-toed sloth, no. 10, normal.
Test, effect of cortico-adrenal extract. 2:15 p.m., walking on pole, 12 foot; time, seconds per 12 foot, 25, 26, 28, 25, 26, 28, 26, 30, 32, 32; average 27.8. Gave 5 cc. cortico-adrenal extract s.c. at 2:25 p.m., 5 cc. at 3:15 p.m. Times of runs, 4:30 p.m., 18, 18, 16, 18, 20, 20, 19, 20, 20, 20; average, 18.9. Further doses of extract given; running time, average 19 sec.
Increase in rate after extract, 31 per cent.

The increase in activity brought about by raising the body temperature, on exposing sloths to the sun, was somewhat greater than under any of the other conditions studied. In 5 of these cases, the rises in rate of running were over 100 per cent above normal. In 17 series of runs, considering both species of sloths, the average rise was 45 per cent (table 1).

The larger increases in activity were usually associated with greater rises in body temperature. In the series of two-toed sloths exposed to the sun for 1 to 2 hours, the body temperature rose from an average of 33.4° to 36.8° (8 cases); in three-toed sloths, from 32.0° to 36.4° (9 cases). In other experiments there were observed rises in rectal temperature usually within 1°, except after prostigmin injection in which case increases up to 2° occurred. The temperature increments in sloths on emotional excitation have already been noted (Britton and Kline, 1939).

There was no significant difference in increases in activity under other conditions studied, in which they approximated 30 per cent. On the

two-toed sloth, however, cortico-adrenal extract did not appear so effective, there being an 18 per cent increase only compared to 32 per cent in the three-toed form.

The greatest effect of emotional excitement was shown immediately after excitation. Adrenalin and prostigmin effects were optimum within 20 to 40 minutes, and cortico-adrenal extract in 2 to 4 hours. In several cases the beneficial action of the adrenal hormone was maintained for 8 to 12 hours. Prostigmin appeared to act somewhat like emotional excitation, and often brought out a strong fighting reaction in the sloth, especially the 2-toed species.

TABLE 1

Increase in activity of sloths under different conditions

(Tests of animals walking up side down on under surface of horizontal 12-foot pole. Each series was made up of 8 to 12 individual "runs." See text.)

ANIMAL	EXPERIMENTAL SERIES	NUMBER OF SERIES OF RUNS	NORMAL RATE	EXPERIMENTAL RATE	INCREASE IN RATE AVERAGE PER CENT
			Av., sec. per 12 ft.		
Two-toed sloth	1. Cortico-adrenal extract injection	6	23	19	18
	2. Adrenalin injection	9	23	15	33
	3. Prostigmin injection	11	35	27	24
	4. Emotional excitement	9	25	16	35
	5. Exposure to sun (warming)	8	29	15	48
Three-toed sloth	1. Cortico-adrenal extract injection	4	31	21	32
	2. Adrenalin injection	8	36	26	27
	3. Prostigmin injection	7	34	21	38
	4. Emotional excitement	15	39	28	29
	5. Exposure to sun (warming)	9	37	21	42

Several other substances were tested for their possible influence on sloth activity—thyroxin and thyroid extract, pituitary extract, benzedrine sulphate and strychnine sulphate. All were without significant effect, however, on the rate of progress of the sloth under the given conditions. Observations after the injection of thyroid preparations were made over a period of three days.

It appeared from over 900 individual tests that the two-toed sloth travels on an average 1 mile in 3 hours, and the three-toed species 1 mile in 4½ hours. Many animals nevertheless take 6 or 7 hours to cover the distance. The rate of progress in the wild is probably much slower. On excitation in the laboratory a mile an hour may be covered by the two-toed sloth, and a mile in 2 hours by the three-toed species. Some years ago the increase in activity brought about by cortico-adrenal extract was

observed in this laboratory (Eagle, Britton and Kline, 1932), and numerous workers have since reported confirmatory results.

SUMMARY

In several hundred tests the sloth was observed to progress on the average at the rate of 1 mile in 3 or 4 hours. Under excitation its speed was increased by 25 to 50 per cent, or in some cases more than 100 per cent. Both two-toed and three-toed species were used; the latter were the slower animals.

Emotional excitement, and raising the body temperature of the sloth, were very effective stimulants to increased rate of movement.

Cortico-adrenal extract, adrenalin and prostigmin also markedly increased bodily activity. The beneficial influence of the cortical hormone was maintained for periods up to 12 hours. Several other substances were found ineffective.

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CONDUCTION VELOCITY AND DIAMETER OF NERVE FIBERS

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Previous studies of the relationship between the size of nerve fibers and the velocity of conduction of impulses have resulted in the bringing forward of three formulations:

1. $V = kD$ (Gasser and Erlanger, 1927; Gasser, 1934)
2. $V = kD^2$ (Blair and Erlanger, 1933)
3. $V = kD^{0.5}$ (Pumphrey and Young, 1938)

At the time at which the present studies were started only the first two proposals had been made, and it was hoped that by the utilization of an experimental method different from those used for the measurements that constitute the basis of the original derivation, new evidence would be acquired which would throw the balance toward one or the other of the two formulas. The method employed depends upon the generally accepted assumption that in any given nerve the largest fiber conducts at the highest velocity. The procedure in accord with the method is to select nerves with different maximal velocities, measure the velocities, and then prepare histological sections of the nerve in which measurements of the diameter of the largest fiber can be made.

A similar method was used by Pumphrey and Young for the small fibers in their nerves. The velocities in the large axons were determined individually and the diameters were measured directly in the fresh state. Squid nerves were used exclusively in their experiments, and this fact must be kept in mind when their data are compared with the data to be presented later, all of which were obtained from medullated vertebrate nerve.

METHOD. In order to obtain valid velocity readings it is essential that the fibers be in the best possible condition. Precaution is especially necessary when small fibers are concerned, as they are more susceptible to injury than large fibers, and if injured they acquire a velocity rating that is too low. The method is a favorable one from the standpoint of the conservation of fibers, as dissection is limited to freeing the nerves of adherent fascia, and it is not necessary to use small strands. Excised nerves of the cat were mounted on silver-silver chloride electrodes in a moist

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chamber, and velocity readings were made as soon as the nerve could be brought into equilibrium with the temperature in the chamber which was maintained at 37.5°C. After the measurements had been made, the nerve was immediately taken out and fixed. Thus distortion of the fibers, which is often seen in nerves that have been subjected to long periods of stimulation, was held to a minimum. Krebs-Ringer solution was used to moisten the nerve during the experiment, and the atmosphere in the observation chamber was 5 per cent CO_2 in O_2.

Measurement of velocity. Condenser discharges controlled by a thyratron were used for stimulation, and the shock strengths were adjusted to give a maximal response of the group of fibers yielding the first elevation in the action potential of the nerve stimulated. Oscillographic records of the action potential were made at five or more distances of conduction, and then the shock-response times were measured on enlarged projections of the records. Because of the utilization period of the shock and the possibility of spread of the stimulus, no single shock-response time can be depended upon for the calculation of the velocity. But the series of times, plotted against the distances, yields a straight line graph the slope of which gives the velocity freed from these sources of error.

High amplification was used for recording. In nerves containing large fibers it was possible, by reducing the strength of shock, to stimulate single fibers and record from them. In view of this fact the conclusion is warranted that the first deviation of the action potential from the baseline in the records made for velocity measurements could be referred to the single largest fiber in the nerve. When small fibers were being studied it was not possible in nerves of the sizes employed to record single spikes with certainty, because of interference by the noise level. Arbitrarily therefore it was assumed, when the maximal velocities were 20 m.p.s. or less, that the first deviation of the action potential should be referred to the three largest fibers instead of to the single largest fiber. Procedure on this basis introduced at most only a small error, since the average diameter of the three largest fibers was ordinarily only a few tenths of a micron less than the diameter of the largest fiber.

Measurement of fiber diameter. When the nerves were removed from the moist chamber they were tied to glass rods to prevent a change in length, and fixed in either 1 per cent osmic acid or 10 per cent formalin. In the latter case they were subsequently stained according to the Kultschitzski technique. The osmic acid method was satisfactory for nerves in which large fibers were to be measured and it had the advantage of being less time-consuming. For nerves presenting small fibers for measurement the second method was preferable. The size measurements, however, were found not to be significantly different when a portion of a nerve prepared in one way was compared with another portion of the same nerve

prepared in the other way. The sections were made after embedding in paraffin, and the diameters of the fibers outside of the myelin sheath were measured with the aid of an ocular micrometer.

Sources of error. 1. *Tapering or branching of the nerve fibers.* The nerves used for embedding were cut into three pieces, and the pieces placed side by side in the paraffin block. Thus each of the finished slides contained in the serial sections samples of the middle segment of the nerve and of the two end segments; and the juxtaposition of the samples made it convenient to determine whether the "largest fiber" tapered or branched in the course of the preparation upon which the velocity measurement had been made. As was to be expected from the straight-line character of the conduction-time conduction-distance curves, no evidence of tapering or branching was found in any of the preparations. It should be stated, however, that the only means of identifying the largest fiber in widely separated nerve regions was by its size, and the measurements reported here establish only the fact that the largest fiber in the peripheral segment of the preparation was not smaller than the largest fiber in the middle and proximal segments.

2. *Random variation of the fiber diameter.* In order to investigate the magnitude of the possible variation in size in the course of the fibers, due to the technique of preparation or other causes, measurements were made of 100 consecutive 6 mu paraffin sections of a saphenous nerve stained with osmic acid. By following one of the small bundles which made up the nerve, it was possible to make sure that the several measurements were all made on the same fiber. The distribution of the measured diameters is shown in figure 1. From the data it can be shown that the standard deviation of a single measurement of a 6.5 mu fiber (modal value) is ± 0.47 mu. As the slides, prepared from the nerves on which the velocity measurements had been made, contained six sections each from three portions of the nerve and as the largest fiber was measured in each of the sections, the error of measurement would be less than that for a single measurement.

If this variation in diameter is brought about by the histological preparation and is not a characteristic of the living fiber, it must occur during the fixation and not in the dehydration process, since Duncan (1934) has shown a comparable diameter alteration along the length of nerve fibers fixed in osmic acid and teased in glycerine.

3. *Differential distortion of large and small fibers.* If nerve fibers of all sizes are not affected in the same manner in the course of histological preparation, the true form of the curve relating velocity and nerve diameter will not be revealed. It was, therefore, necessary to show either that distortion does not occur during the preparation of the nerves, or that if it does occur, it affects fibers of different diameters in a proportional manner.

Donaldson and Hoke (1905) reported that in nine experiments on the

sciatic nerve of the rat there was an average increase in nerve diameter of 0.3 per cent in the course of the steps from the fresh to the paraffin embedded state. They measured the diameter of the entire nerve trunk, as they believed it was impossible to tease out single fibers without stretching them and altering their reactions to histological reagents. Lapicque and Desoille (1927) measured the ranges of variation in the diameter of the largest fibers found at the several levels of the frog sciatic nerve. The range at a given level was the same whether the diameters were measured on fresh teased fibers or on the paraffin cross-sections of fixed specimens.

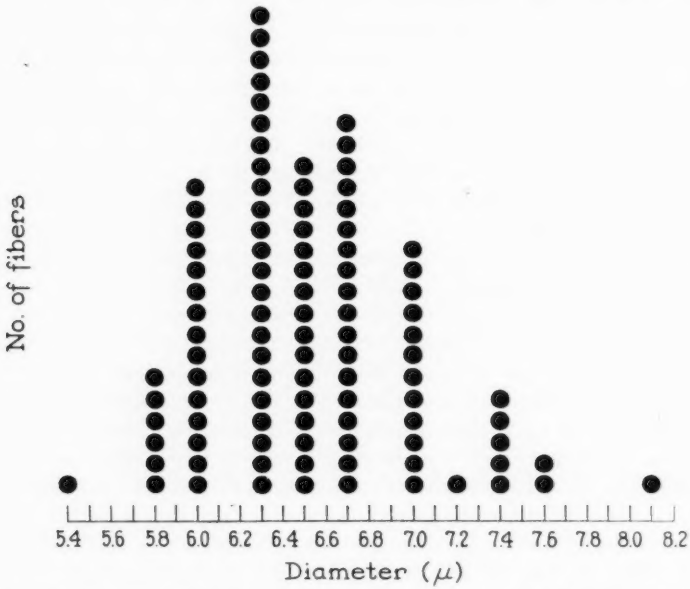

Fig. 1. Distribution of the diameter measurements on a single fiber followed through 100 six-mu serial sections.

Duncan (1934) also examined the question of shrinkage and concluded that the diameters of the living fibers were retained in the mounted cross-sections.

Evidence to the contrary has been presented by Arnell (1936) who measured the fiber diameters in fresh frozen sections and in paraffin sections of the same nerve. Comparison of his tables II and XII shows that a shrinkage of 20 to 30 per cent occurred during the histological preparation. Our experience is in agreement with that of Arnell in showing that shrinkage is produced.

Since the difficulties of dealing with single fibers were recognized, small

spinal root bundles having relatively little connective tissue were used as experimental material. Bundles measuring 0.1 to 0.05 mm. in diameter were attached with ceresin to a glass slide. The slide was ruled at right angles to the course of the nerve, so that measurements could be repeated at exactly the same locus. Diameters at eight points along each bundle were measured by means of a binocular microscope fitted with an ocular micrometer. Fixation in osmic acid caused an average (36 nerve bundles) increase in diameter of 2.2 ± 0.5 per cent. Fixation and dehydration caused an average decrease in the fresh nerve bundle diameter of 23.6 ± 1.2 per cent.

The interpretation of these results was made somewhat ambiguous by the fact that although the spinal root bundles do not have the thick outer connective tissue sheath found in nerve trunks, there is connective tissue between the individual nerve fibers. Doubt, therefore, existed regarding the proportion of the total shrinkage due to connective tissue as compared with that due to nerve fibers.

Inasmuch as fixation in osmic acid did not change the root bundle diameter, it was thought probable that the nerve fiber was not distorted by this treatment. Nerve fibers were, therefore, teased out from a spinal root bundle which had been fixed in osmic acid. Measurements of the nerve diameters were made before and after dehydration. Twenty measurements were made for each diameter and eleven nerve fibers, ranging in size from 22.5 to 10.2 mu, were examined. The shrinkage found varied from 8.2 to 12.9 per cent, with an average value of 10.1 ± 0.16 per cent. There was no correlation between the percentage of shrinkage and the size.

These data considered in relation to the nerve bundle experiments show that a considerable proportion of the shrinkage found during dehydration is due to connective tissue. Shrinkage of the fibers occurs, however, but without indication of its being differential.

Nerves used in the investigation. The maximal velocity of conduction and the diameter of the largest fiber were measured in the following nerves of the cat:

NERVES	MAXIMAL VELOCITIES
	m.p. sec.
Peroneus	108–111
Suralis	69– 86
Saphenous	65– 82
Vagus	67– 73
Cervical sympathetic	29– 56
Hypogastric	11– 41

RESULTS. The data obtained from these nerves are presented in figure 2. The points seem to fit a straight line more readily than any other

graph. The line which has been drawn is the calculated regression line. It has a slope of 6.0, which means that the velocity in meters per second can be obtained by multiplying the diameter in micra by this factor. The broken lines represent the velocity as a function of the square and the square root of the diameter respectively, assuming that a 13.8 mu fiber conducts at a velocity of 78 meters per second. It is quite apparent that the data are not compatible with either of these two assumptions; and they would be still more incompatible if the square and the square-root

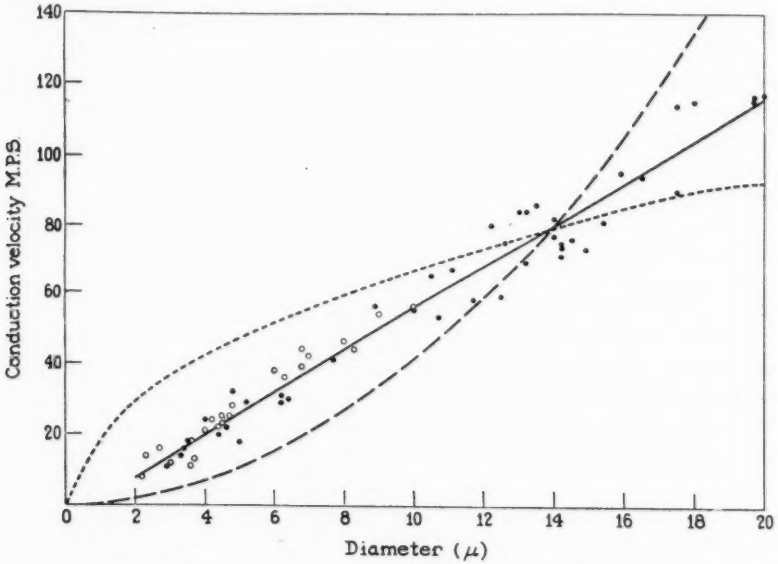

Fig. 2. The fastest conduction rate plotted against the diameter of the largest fiber for nerves of different velocities. The dots are data on adult cats; circles, data on kittens. The dotted and dashed lines represent the velocities calculated as the square root and square functions of the diameter.

curves were drawn to include the points representing the extremes of velocity.

The diameters of the constituent fibers in any particular nerve are known to be smaller in young animals than in the adult (Boycott, 1904; Duncan, 1934). Young animals, therefore, provide an additional source of nerves in which the largest nerve fibers are of small caliber. On these nerves two questions may be studied. Is the conduction velocity in immature nerves proportional to the diameter and is the factor of proportionality the same as that holding for adult fibers?

Both questions may be answered in the affirmative. The circles in

figure 2 present data obtained from the nerve trunks of kittens. It is obvious that they lie very close to the regression line drawn for the cat fibers. The exact slope of the latter is 6.04. The regression line drawn for both the kitten and cat fibers has a slope of 6.00.

DISCUSSION. Correlation of the conduction velocity with the outside diameter of the fiber means that the velocity may be determined by any

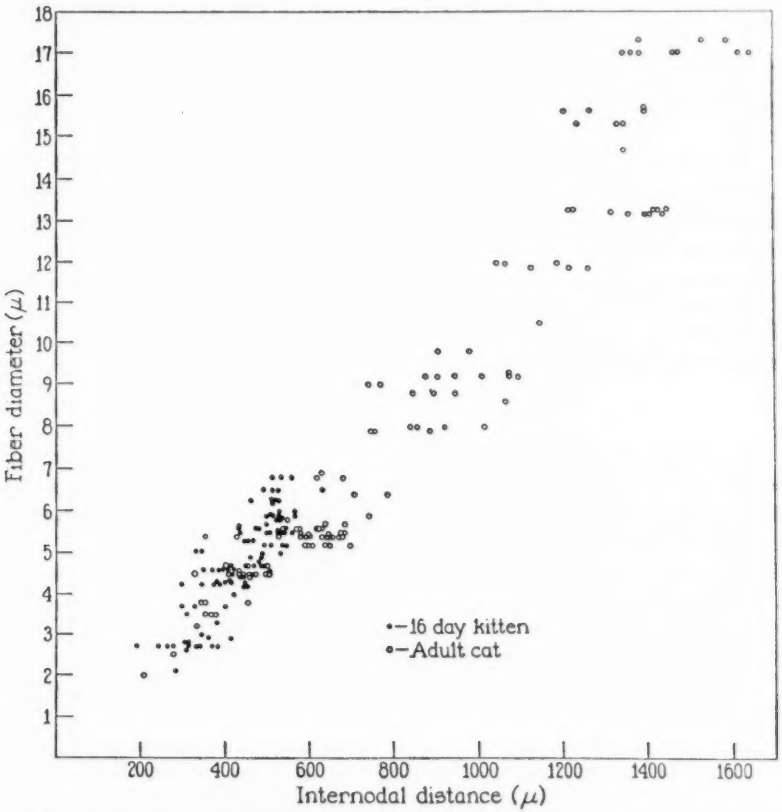

Fig. 3. The internodal distance plotted against nerve fiber diameters of a 16-day-old kitten and an adult cat.

dimension in linear relation to the diameter. The axon diameter is the dimension demanding first consideration. According to Donaldson and Hoke, the ratio of the axon diameter to the total diameter is constant. On the other hand, Arnell (1936) and Schmitt and Baer (1937) find a systematic deviation in the ratio dependent upon the fiber size. The scatter in

the data, however, is too great to permit their use for or against an hypothesis proposing control of the velocity by the axon diameter.

Another dimension to be considered is the length of the internodal segments. The evidence that these lengths are proportional to the fiber diameter has recently been cited by Zotterman (1937). Before the internodal lengths could be considered, however, it would be necessary to show that the lengths are the same in kitten and in cat fibers of the same diameter, inasmuch as kitten and cat fibers of the same size conduct impulses at the same velocity (fig. 2). The point has been especially examined because Boycott in 1904 published data showing that in frog fibers of a given size the internodes are longer in mature frogs than they are in young frogs.

The fibers of the peroneal nerve were teased in a 50 per cent glycerine solution, after fixation with osmic acid according to a method described by Takahashi (1908). The data are presented in figure 3. The dots represent kitten fibers and the circles cat fibers. In the region where the diameters overlap there is very little difference between the internodal lengths for the two ages. The relationship defined by the curve, therefore, is consistent with the substitution of internodal length for the fiber diameter in arguments about the control of velocity.

SUMMARY

The conduction velocities in the fastest fibers in various nerve trunks of the cat and the kitten were correlated with the outside diameters of the largest fibers found in those trunks. The limits of the range of velocities measured were 8 and 117 meters per second.

The best curve relating velocity and diameter is a straight line. It holds equally well in all parts of the range of velocities. Curves drawn in accord with the hypothesis that the velocity varies as the square or square root of the diameter vary widely from the observed points.

Comparative data relating internodal length to fiber diameter are plotted for nerve fibers from cats and kittens.

The author is deeply indebted to Doctor Gasser for suggesting the problem and for his helpful criticism during the course of the work.

It is a pleasure further to acknowledge the advice and assistance of Doctor Grundfest, and to thank Doctor Lorente de Nó for his supervision of the histological technique.

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THE PROPERTIES OF GROWING NERVE FIBERS

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The research here described deals with the changes that take place in the nerve fibers of kittens during the period of growth. Preparations of the saphenous nerve and of the cervical sympathetic trunk were made from kittens ranging in ages from a few days to three months. After the age of three months the properties do not differ essentially from those of corresponding nerves of the adult cat. On these preparations, determinations were made of the velocity of conduction, of the spike duration, the refractory period, and the configuration of the after-potentials. Correlation of the velocity with the diameter of the fibers will be reported in another connection. For the present it may be stated that the velocities of kitten fibers ranging in diameter from 4 mu to 10 mu have been measured. When the velocities and the corresponding diameters are plotted, the points are randomly distributed about a straight line.

In the following description it will be necessary to mention three groups of fibers—A, B, and C. A refers to the myelinated fibers of somatic nerves and to fibers in visceral nerves having similar properties. In sensory nerves the fraction of these A fibers that gives the elevations conducted at about 20 m.p.s. is called delta. B refers to the group labelled B_2 by Bishop and Heinbecker. It is found only in visceral nerves and is made up of fibers having in the adult cat velocities between 15 and 4 meters per second. C refers to the unmyelinated fibers, velocities 2 m.p.s. and slower.

Since, during the period of growth, as much as a sixfold increase takes place in the velocities of the A and B fibers, opportunity is afforded to ascertain the extent to which these groups possess their distinguishing characteristics during the stage in their development at which the velocity of conduction is that of the next slower group. The specific question is asked: Do the spikes, refractory periods and after-potentials in fibers, which in the adult will be called A fibers, have the properties characteristic of A fibers or B fibers, when the fibers conduct impulses at B velocity? And a similar question is asked with respect to immature B fibers conducting at C velocity.

Records were made with a direct-coupled amplifier designed by Doctor

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Toennies. In order to make the spike monophasic, the nerve trunk was in all cases crushed between the two lead electrodes. Despite the exercise of care in removing pieces of connective tissue from the nerve stretch between the electrodes, so as to avoid capacity effects to ground, and in killing the nerves just before the records were taken, a certain amount of diphasicity is to be observed in some of the records. This difficulty, however, does not interfere to any large extent in the interpretation of the recorded findings. Stimulation was effected by short thyratron discharges applied to the nerve through a transformer. At the conclusion of the experiment certain of the nerves were fixed in osmic acid or formalin and paraffin sections were prepared.

Change of the spike potential with growth. The saphenous nerve. Figure 1 shows spike potentials recorded from saphenous nerves of kittens 4 to 78 days old. Between these ages the velocity of conduction in the fastest fibers increases progressively from 11 to 60 m.p.s. Since all kittens do not grow at an equal rate, better correlation might be expected if some measurement of growth other than age were compared with velocity. The length of the kitten's leg from the head of the femur to the tip of the toes was the measurement selected. In figure 2 these lengths are plotted against the fastest velocities in the corresponding excised saphenous nerve trunks. The relationship as shown here is clearly a linear one.

Two generalizations may be made on the basis of the relationship. First, as far as peripheral nerve is concerned, the movements of the kitten are not slowed because of the decreased conduction rate, since the latter is nicely compensated for by the decreased length of the nerve path. Secondly, it may be inferred that the fibers grow in length and diameter at an approximately equal rate, as the diameter is proportional to the velocity.

In the growth of the saphenous nerve there occurs an early division into the velocity groups that are characteristic of the adult nerve. The record of the 8-day nerve of figure 1 shows the delta group represented as a small elevation conducted at 4 m.p.s. In records taken at various stages of the subsequent growth of the nerve, the delta fibers regularly appear as a distinct velocity group. Frequently there occurs a separation of the faster A fibers into two velocity groups, which gradually merge as the nerve becomes adult.

The cervical sympathetic nerve. Figure 3 shows the potential records at several stages in the growth of that portion of the cervical sympathetic nerve trunk included between the superior and middle cervical ganglia. In order to show fast and slow groups clearly, several sweep speeds and amplifications were employed for recording the 20- and 90-day nerves. The 90-day nerve represents a picture that falls within the range of variation found in the adult nerve: a small fast group of A fibers (32 m.p.s.), a second large group of B fibers (15 m.p.s.), and a slow group of C fibers

(1.7 m.p.s.). In contrast to this picture, the records of the 5- and 8-day nerves show a *single* elevation conducted at velocities typical of adult C fibers. The failure of differentiation into separate velocity groups is

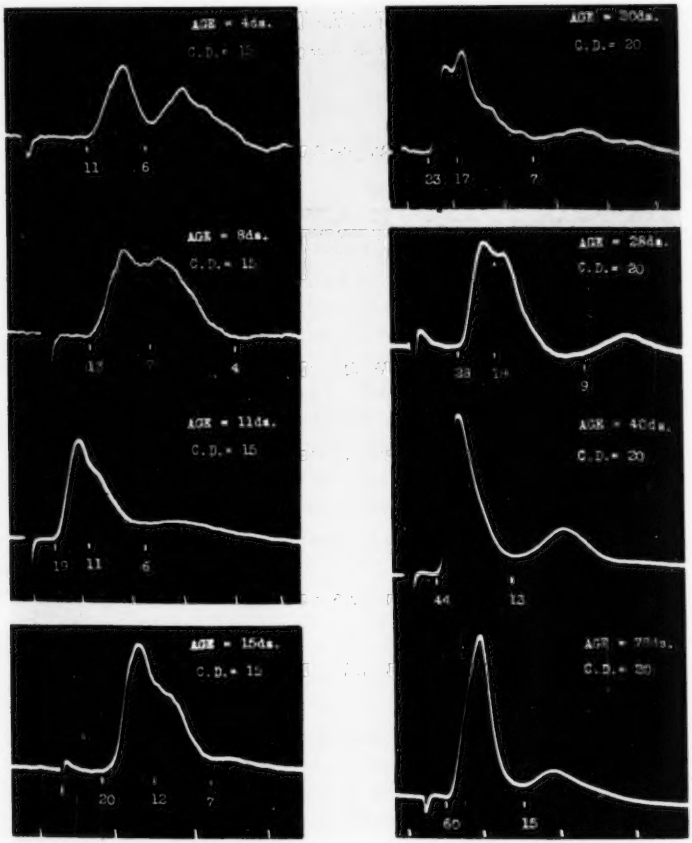

Fig. 1. Changes in the saphenous nerve spike potential during the growth of the cat from 4 to 78 days. The numbers along the bottom of the photographs give the velocities of the denoted groups in meters per second. The pictures are grouped according to the speed of sweep which is noted in msec. at the bottom of the respective groups. The shock was slightly supermaximal.

maintained up to an age of about 12 days, unless depressor fibers are included in the nerve stretch examined. (See table 1, expts. 3, 7 1, 8 1, 10 1.)

Billingsley and Ranson (1918) have described in cats a depressor branch

sometimes observed separate from the vagus in the carotid sheath. Bishop and Heinbecker (1932) regard as afferent vagus fibers the fast group (their S_1), which they usually record ahead of the standard elevations of the cervical sympathetic nerve of the adult cat.

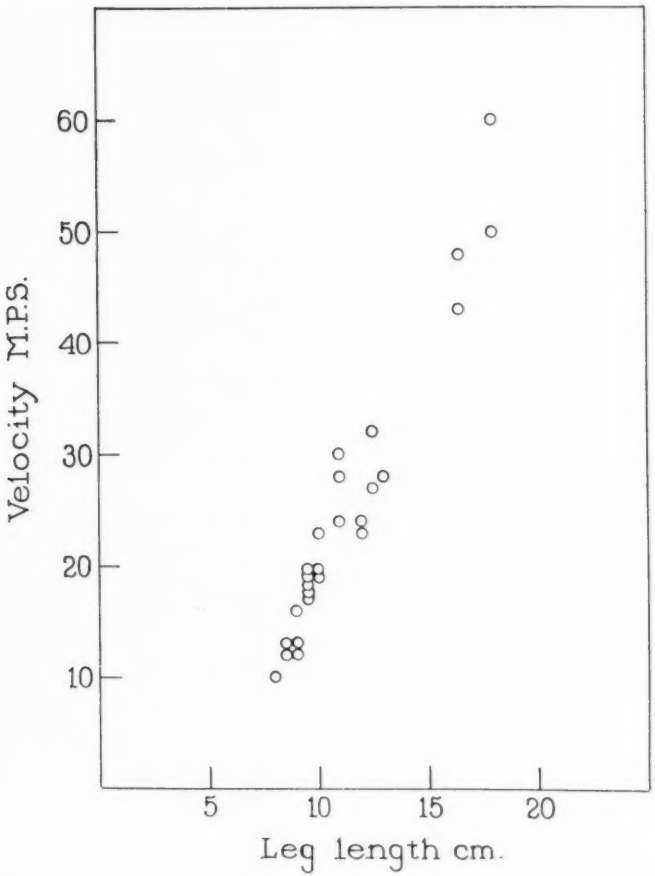

Fig. 2. The relation between the velocity of the fastest fiber in the saphenous nerve and the corresponding length of the kitten's leg, from the head of the femur to the tip of the toes, for leg lengths representing different stages of growth.

In a series of eight experiments performed by the author on adult cats, in a single case, stimulation of the cervical sympathetic nerve brought about a lowering of blood pressure.

In the exceptional cases that are cited above as showing a separate

velocity group preceding the main slow wave, a small branch was found accompanying and frequently joining the cervical sympathetic nerve.

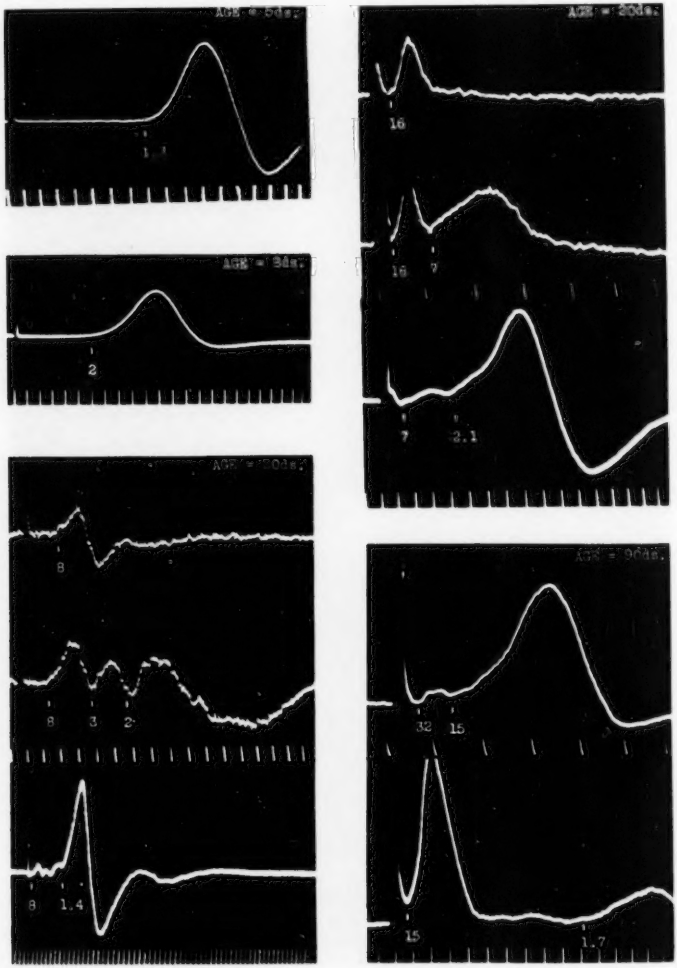

Fig. 3. The cervical sympathetic nerve spike potential at growth stages as labelled. Velocities are noted below the photographs in m.p.s. The 20-day nerves are from the right side (lower set of records) and the left side (upper set) of the same animal. Sweep speeds are in msec.

This branch, when separate, could be dissected back to the nodose ganglion. When the branch and the cervical sympathetic were excised and stimulated

TABLE 1

Growing cervical sympathetic nerve

EXPERIMENT	AGE	VELOCITY GROUPS† AND REFRACTORY PERIODS‡		
	days			
1	4			2.3 (3.0)
2	5			1.3 (2.0)
3	5		7.6* (1.4)	1.6 (3.3)
4 l	5			1.0
4 r	5			1.0 (3.1)
5	8			2.0
6 l	8			1.0
6 r	8			1.8 (3.0)
7 l	8		10.0*	1.6
7 r	8		(1.4)	0.5
8 l	9		6.0*	1.1 (2.6)
8 r	9			1.0 (2.7)
9 l	11		2.2 (2.0)	0.7
9 r	11		2.3 (2.5)	1.5
10 l	11		8.5* (1.1)	1.4 (2.5)
10 r	11			1.3
11 l	12		3.4	1.3
12 l	14		3.3	1.3
12 r	14		3.3	1.1
13	15		1.1 (2.0)	0.6
14 l	16		6.0	1.1
14 r	16		5.7	1.1
15 l	17	15	8.0	2.0 (2.3)
15 r	17		8.6 (1.2)	1.7
16 l	20	16 (1.0)	6.5 (1.5)	2.2
16 r	20		7.6	2.0
17	28		7.5 (1.6)	2.0
18	40	25	10.0	1.7
19 l	60	28	9.0	2.3
19 r	60	18		1.6
20	78	28	12.0 (1.2)	1.7

Experiments marked l and r are nerves from the same animal (l is left side).

* Groups marked in this way are afferent vagus fibers.

† Where clear separation into different velocity groups is found, the fastest velocity of the different groups is noted in m.p.s.

‡ Refractory periods are enclosed in parentheses and are expressed in msec.

separately, the branch potential contained the fast group and the cervical sympathetic only the slow wave.

It is, therefore, concluded that the A fibers of the depressor branch account for the small elevation occasionally found preceding the slow wave in records of 4- to 12-day cervical sympathetic nerves. If at this stage of growth any cervical sympathetic A fibers exist as a separate group, the corresponding spike potential cannot be seen.

Since all cervical sympathetic nerves have a large group of B fibers, it is of interest that no corresponding elevation can be seen in these immature nerves. The possibility that the preganglionic B fibers are not functional, and hence do not contribute to the potential, is negated by the following experiment. An 8-day old kitten was anesthetized with dial and the cervical sympathetic nerve was dissected from the vagus and the depressor trunks. The nerve was cut near the middle cervical ganglion, and the preganglionic fibers leading to the superior ganglion were stimulated. An immediate enlargement of the pupil occurred. The preganglionic nerve was then excised and mounted in a moist chamber for the recording of its action potential. A single elevation, in which the fastest fibers were conducting at 1 m.p.s., was found. If these pupillo-dilator fibers which are functional in kittens are the same as those functioning in the adult cat, they will develop to constitute the fastest part of the B group, as Bishop and Heinbecker (1932) have shown that the pupillary reaction is mediated by fibers the activity of which is manifested in this portion of the action potential.

After the first 12 days, during which the fibers producing the slow wave show no demonstrable increase in velocity (table 1), the action potentials of a small disperse fiber group appear ahead of the main potential elevation. These potentials subsequently increase in size because of the appearance of additional fibers in the group and the growth of those already present. The constituent fibers also increase in velocity. At 20 days the B group is definitely set off from the C group; and ahead of the B group an A group is usually visible.

The saphenous nerve. Effect of growth on the positive after-potential. When the delta component of the A group of fibers first appears, the fastest fibers in the group have a velocity characteristic of B fibers (4–5 m.p.s.). This fact makes it possible to investigate an important point with respect to the after-potentials. At this early stage, are the after-potentials those characteristic of A fibers, in which case one would take them to be determined by the kind of fiber? Or, are they characteristic of B fibers, in which case one would take them to be determined by the size of the fiber?

The method of procedure was to make records of the action potential as excited by two strengths of shocks—the first shock strong enough to

stimulate only the fast group of fibers, and the second shock strong enough to stimulate both groups. A complete separation of the groups could not always be effected, as some late group fibers were stimulated before the quota of early group fibers was complete, but this difficulty was not sufficiently great to destroy the experiments. In these records, the sizes of the after-potentials were measured, directly on the first group and by difference on the second group. The sizes were then compared with the height of the corresponding spike, corrected for temporal dispersion. The correction was made by calculating the height of a spike of 0.5 msec. duration[2] which would have an area equivalent to the area of the spike complex in the record as measured with a planimeter.

The results of 10 experiments are presented in table 2. These experiments were chosen from a larger number because of the relative absence of diphasicity in the potential records. Diphasicity would upset the after-potential spike-height ratios by making the area of the spike too small and the size of the after-potential too large.

Before examining the table it will be necessary to set forth the dimensions of the positive after-potentials (p.ap.) in A and B fibers. In A fibers the p.ap. ends at 60–80 msec., and its amplitude at the maximum is 0.1–0.4 per cent of the spike height (Gasser and Grundfest, 1936, p. 116). In B fibers the p.ap. ends at 100–150 msec., and its amplitude is 1.5–4.0 per cent of the spike height. (Grundfest, unpublished data.)

The last column in table 2 shows that the p.ap. in immature delta fibers has the properties characteristic of A fibers. The duration is an A duration and the size, while somewhat larger than that associated with the fast A group of the same nerve, is close to A dimensions and far from B dimensions. In one instance only does the size approach a B size (the 8-day nerve) and in this case the duration is distinctly that of an A fiber.

Single spike durations and refractory periods. Table 3 gives the values of single spike durations and refractory periods for the saphenous nerves. The technique employed to record the single spike durations was to use high amplification and to adjust the shock strength at just threshold for the most excitable fibers. As a result, the information obtained concerns only the spike durations of the fastest fiber (strictly the most excitable fiber) in any mixed nerve trunk. It is known that the potential recorded from a nerve diminishes with fiber velocity. Accordingly, a natural

[2] In this calculation it is assumed that the immature delta fibers have a spike duration of 0.5 msec. Table 3 lists spike durations of 0.60 to 0.70 msec. for A fibers conducting at velocities from 12 to 9 m.p.s. These are average values, however (see following text), and show a range of variation from 0.4 to 0.72 msec. As this evidence for a longer duration is regarded as inconclusive and as the other A spike durations listed in table 3 are independent of conduction velocity, the above assumption may be considered reasonable.

limitation to this method of measuring single spike duration is reached when the amplitude of the recorded spike becomes of the same order as the noise level of the amplifier. The determinations of single spike durations given in table 3 are, therefore, more accurate in the case of the fast than of the slow fibers. The customary procedure was to photograph about 50

TABLE 2

The positive after-potentials of saphenous nerve fibers at various stages of growth

AGE	VELOCITY GROUPS		POSITIVE AFTER-POTENTIAL		
	Weak shock m.p.s.	Strong shock m.p.s.	Duration msec.	Amplitude	
				μv.	Per cent*
days					
8	15		48	12	0.4
		9–5	40	51	0.9
17	18		60	4	0.21
		8.5–5.3	70	7	0.47
19	19		70	8	0.49
		9.3–3.5	85	13	0.45
19	17.5		70	7	0.43
		8.7–5.7	70	7	0.56
20	23–6		60	32	0.50
24	32		62	18	0.20
		8.1–3.0	80	18	0.58
28	32		55	23	0.52
		7.5–3.8	55	16	0.57
28	27		65	10	0.31
		14.3–8.4–4.5	65	17	0.38
40	48		55	14	0.17
		15	67	3	0.10
78	60–15		50	40	0.3

* Amplitude at the maximum calculated as percentage of the height of a 0.5 msec. spike having an area equivalent to that of the recorded spike.

threshold responses. From this group the 10 best examples were selected as those showing the least noise interference at the beginning and the end of the spike. The averages of the durations of these spikes are presented in the table.

The spike durations in table 3 fall within the range of values (0.4–0.5 msec.) that has been determined for the faster fibers of the A group. The

TABLE 3

Growing saphenous nerve

AGE	VELOCITY GROUP	REFRACTORY PERIOD	S.F.S. DURATION	AGE	VELOCITY GROUP	REFRACTORY PERIOD	S.F.S. DURATION
days	*m.p.s.*	*msec.*	*msec.*	*days*	*m.p.s.*	*msec.*	*msec.*
2	10	0.70	0.67	17	28		
	3				8		
					5	1.05	
2	14	0.65	0.64				
	3			17	19	0.56	
					9	0.80	
3	9	0.60	0.70				
	4			17	18	0.55	
					9		
					6		
3	14	0.60					
	4			19	19	0.58	
					7		
5	12	0.65	0.60				
	7			19	18	0.58	
	5				9		
					6		
5	13	0.76					
	8			20	23	0.37	
					10		
5	12	0.75			5		
	8						
				27	20	0.46	
9	12	0.75			7		
	4						
				27	23	0.49	
11	19	0.51	0.45		8		
	7						
	5	1.20		28	28	0.53	0.53
					8		
11	19	0.53	0.55				
	9			28	32	0.50	
	5	1.10			8		
11	12	0.85		28	27	0.51	
	7				14		
	4				8		
11	12	0.78		40	44	0.43	
	4				14		
15	20	0.43	0.50	78	60	0.40	0.45
	7				15		
17	24	0.53	0.45				
	7						

first four determinations are made on fibers of low velocity and come within the category referred to above as being less accurate. For this reason some doubt exists that the slightly increased spike durations are significant.

For measurement of the refractory period the conditioning shock was made slightly stronger than that necessary for a maximal nerve response, and the testing shock was increased to three times this value. In a nerve trunk in which the velocities of the component fibers vary regularly, it is clear that the refractory period of only the fastest fibers could be measured. Where there is a clear separation into velocity groups, as in the case of the saphenous nerve, determinations can be made for the fibers heading each group. Thus in table 3, absolutely refractory periods are listed for the delta fibers as well as for the faster A fibers.

The absolutely refractory periods of the most rapidly conducting fibers in growing nerves vary between 0.38 and 0.53 msec. when the velocity is greater than 20 m.p.s. Below 20 m.p.s. in the delta range of adult fibers the refractory period is 0.6 to 0.75 msec. Thus the refractory periods are the same as those of adult fibers conducting at the same velocity. Immature delta fibers have velocities below the range obtaining in the A group of the adult nerve and longer refractory periods. In table 3 it is seen that delta fibers of the 11 and 17-day nerves have absolutely refractory periods about twice that (0.65 msec.) of the fastest adult delta fibers.

The cervical sympathetic nerve. Effect of growth upon the positive after-potential. As immature B fibers conducting at C velocities do not exist as a separate velocity group, and as there is no evidence that they respond to a shock strength lower than that of the C fibers recorded as part of the same potential elevation, it was impossible to obtain an independent record of the after-potential of the immature B fiber.

A maximal shock was, therefore, used and the after-potential recorded following stimulation of both the immature B and C fibers. The proportion of B fibers in the adult cervical sympathetic nerve is high. For example, a fiber count of a cervical sympathetic nerve made by Douglas, Davenport, Heinbecker and Bishop (1934) showed 2005 myelinated fibers, predominately B, as compared with 1663 unmyelinated fibers. It is to be expected that the proportion of B fibers in the young nerves would be the same and that these immature fibers would contribute an important part to the composite after-potential recorded. If the contribution is in accord with the velocity of conduction in the fibers, which has a value typical for adult C fibers, the combined after-potential should resemble in all respects the after-potential of adult mammalian C fibers. Table 4 shows that this is not the case.

The combined potentials have the duration of a C fiber potential with the p.ap. lasting more than a second. But the resemblance breaks down

at that point, as the n.ap., which is regularly found in C fibers, is often either absent (expts. 1, 2 and 5) or reduced (expt. 3) in the combined potential. An explanation of the configuration would be afforded by the assumption that the immature B fiber potentials have a quality resembling that of adult B fibers. The large p.ap. would then synchronize with the n.ap. of the C fibers in the combined potential and tend to cancel it out.

DISCUSSION. The questions that were proposed at the beginning of the paper have been answered in part. A fibers conducting at B velocities display what are essentially A after-potentials. But all that can be said about B fibers conducting at C velocities is that they do not display C after-potentials. This meagre information is not without significance, however, as it indicates that although the configuration of the after-potential in B fibers at the stage at which they conduct impulses at C velocity is unknown, it at least has characteristics which are determined to some extent by the kind of fiber and not completely by the velocity. When brought into relation with the fact that the after-potentials in A fibers conducting at B velocities closely resemble those in the adult fibers, the generalization seems justified that the nature of the after-potentials is determined in part at least by the grouping to which the fibers belong.

TABLE 4

Cervical sympathetic after-potentials

AGE	VELOCITY RANGE	POSITIVE AFTER-POTENTIAL			NEGATIVE AFTER-POTENTIAL	
		Maximal amplitude	Time to maximum	Total duration	Duration	Maximal amplitude
days		*μv*	*msec.*	*msec.*	*msec.*	*μv*
5	1.1–0.7	95	150	1,100		
5	1.1–0.7	102	150	1,100		
5	8*,1.6–0.7	25	152	1,000	75	8
9	1.0–0.5	48	200	1,200	90	230
11	8*,1.4–0.3	111	92	900		
17	9–0.4	316	75	400		
60	18–5	42	40	120		

* Small group of depressor fibers.

In contrast to the after-potential, the absolutely refractory period of nerve fibers is not determined by the group to which the fiber belongs. All the measurements of refractory periods that have been made upon kitten fibers are plotted as ordinates in figure 4, with the corresponding velocities as abscissae. In this graph the dots represent A fibers and the circles B fibers. The principal conclusion that can be drawn from these data is that immature A fibers may have refractory periods as long as adult B fibers (1.0–1.2 msec.), and that B fibers conducting at C velocities

may have refractory periods as long as or longer than adult C fibers (1.8–2.0 msec.).

A curve drawn through the points plotted in figure 4 bears a marked resemblance to a comparable curve constructed from the data given by Erlanger (1937, p. 48; see also Blair and Erlanger, 1933) for adult frog

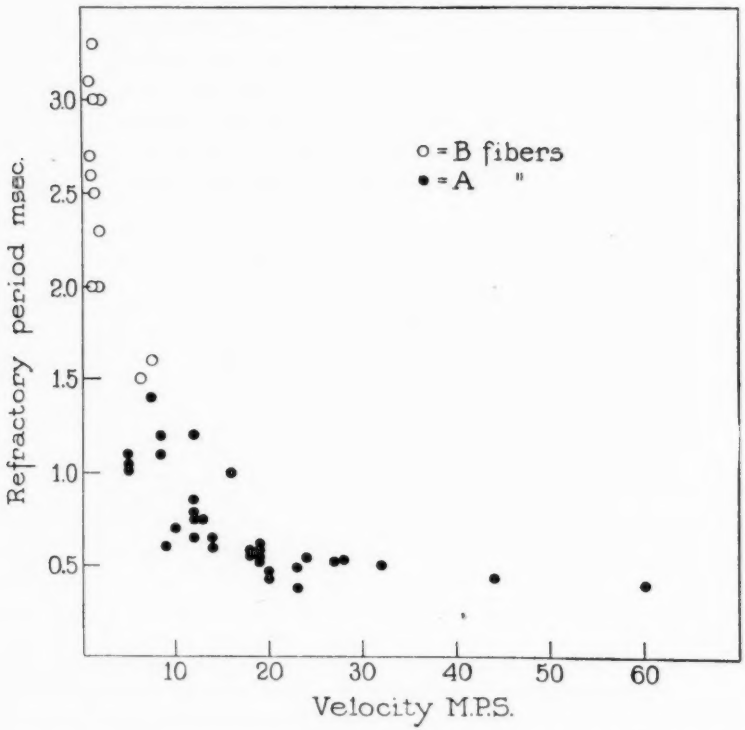

Fig. 4. The refractory periods of immature saphenous and cervical sympathetic nerve fibers plotted against the corresponding velocity of conduction. The dots are data from immature saphenous fibers, the circles from immature cervical sympathetic fibers.

fibers. A single exception to the similarity between the curves is the fact that the kitten fibers show a slight and somewhat questionable increase in refractory period in the range from 60 to 20 m.p.s., while the refractory period of the frog fibers increases more than twofold over the analogous velocity range.

SUMMARY

1. The saphenous and the cervical sympathetic nerves of the cat were studied during their growth.

2. Saphenous nerves of kittens four days old show the A and C elevations in the action potential. As the A fibers grow, their conduction velocities increase. The maximal velocities increase in proportion to the length of the leg.

3. Cervical sympathetic nerves show a single elevation at C velocity in kittens four days old. Differentiation into faster groups starts at about the twelfth day, and the A and B groups are definitely organized at the twentieth day. After the groups appear, the velocities in the constituent fibers increase until adult status is reached.

4. Immature A fibers conducting at B velocities have a positive after-potential with a duration characteristic of A fibers. The amplitude is slightly greater than that characteristic for A, but less than half that characteristic for B. Immature B fibers conducting at C velocity have a positive after-potential differing from that of adult C fibers, but its exact configuration cannot be determined. The after-potentials appear to depend more upon the type of fiber than upon the fiber size.

5. When the absolutely refractory period determinations made upon growing A and B fibers are plotted against the corresponding velocities, the values appear to scatter about a continuous curve irrespective of fiber type. From 60 to approximately 20 m.p.s. the curve displays a slight increase of refractory period from 0.40 to 0.45 msec. At about 20 m.p.s. the increase in refractory period becomes abrupt, so that at a velocity of 10 m.p.s. an average value of about 1 msec. and at 1 m.p.s. an average value of about 2.6 msec. are found.

The author takes pleasure in acknowledging his gratitude to Dr. Herbert S. Gasser and to the members of his laboratory for help and encouragement during the course of this work.

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CERVICAL LYMPH PRESSURE IN THE DOG[1]

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The literature contains only a few reports on lymph pressure in cervical lymphatic vessels. Noll, in 1850, cannulated cervical lymphatics in dogs and found lateral pressures from 0.8 to 1.8 cm. of soda solution. The pressure decreased during inspiration, increased slightly during expiration, and was markedly increased with generalized muscular movements. Weiss (1861) placed a three-way cannula in the cervical duct of several colts and of two dogs under the influence of opium and chloroform. The lateral pressures obtained while the dogs were quiet ranged from 0.5 to 2.0 cm. of a soda solution having a specific gravity of 1.080. These values correspond therefore to 0.54 and 2.16 cm. of water. Weiss noted also that respiration and muscular movements influenced the pressure.

The question was apparently not reinvestigated until 1935 when Rouvière and Valette, by means of a pneumatic cushion placed directly on the cervical duct, measured the pressure necessary to flatten the vessel and to stop the flow of lymph. In dogs with the head immobile they obtained pressures from 1.5 to 2.0 cm. of water. The pressure increased from 4 to 6 cm. when the tongue was passively moved in and out of the mouth.

For peripheral lymphatics, such as those of the limbs, muscular activity is of prime importance, there being very little lymph flow and a negligible pressure during its absence (Field, Drinker and White, 1932). Thoracic duct pressure is influenced by intrathoracic negative pressure, movements of the viscera, and to a limited extent by skeletal muscle activity. The flow and pressure in cervical lymphatics depend on both intrathoracic and skeletal muscle influences. Factors such as venous congestion and the local application of heat, which Landis and Gibbon (1933) found increased the rate of capillary filtration and therefore might influence lymph pressure, have not been examined.

A procedure recently used in this laboratory (McCarrell, 1939) has made

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possible a study of cervical lymph pressure under very constant experimental conditions. A continuous flow of cervical lymph is produced in an anesthetized dog by regular passive motion of the head, and the effect of local heating can be determined by simultaneous irrigation of the nasopharynx with warm saline solution. Lateral cervical lymph pressures under constant conditions of lymph flow were measured in 6 dogs; the effect of venous congestion on lymph pressure determined in 5 dogs; the effect of venous congestion during local heating and the maximum lymphatic end pressure were studied in 2 dogs.

METHOD. Dogs were anesthetized by an intraperitoneal injection of nembutal, and the right and left cervical lymphatics exposed. The larger of the two vessels was cleared of connective tissue for a distance of 4 to 5 cm. and the two horizontal arms of a T-cannula were tied into its lumen. The perpendicular arm of the cannula was attached by rubber tubing to a glass manometer, and the entire system was filled with heparin solution (sp. gr. 1.004). The cannula caused no obstruction to lymph flow and the pressures, corrected for the zero point on the manometer, are believed to be identical with the lateral pressures of the lymph flowing through the cervical duct. Additional heparin, to take the place of that washed away by the lymph flow, was added frequently by injection through the rubber tubing.

The snout of the dog was attached to a crank on an electric motor and with each rotation of the crank the neck was slowly flexed and extended; thus a continuous flow of lymph was produced without the use of massage. Lymph flow in milligrams per minute was determined from the opposite cervical lymphatic cannulated in the usual manner. Each animal received an intravenous injection of saline (20 cc. per kilogram) to provide an adequate supply of fluid. In two cases local heat was applied to the nasopharyngeal region by irrigation with saline at 45°C. The details of these procedures have been reported previously (McCarrell, 1939).

Venous congestion was produced by occlusion of the external jugular veins for a period of 20 minutes, and venous pressures were measured on a saline-filled manometer one arm of which was attached to a needle plunged into the lumen of the external jugular vein.

RESULTS. *Normal cervical lymph pressure.* Pressures recorded during passive motion of the head were generally slightly higher than those obtained with the head quiescent, although this was not true in all cases (see fig. 1). Pressure values to be reported were measured with the head in motion and thus were obtained under standard conditions of lymph flow.

Table 1 summarizes the normal cervical lymph pressures measured during each experiment. The extremes in lymph pressure were found to range from −2.8 cm. to +3.2 cm. of water, with the majority being between +0.1 and +1.4 cm. of water. One animal (exp. 2) in which

the respiratory movements were extremely deep and rapid, exhibited a negative pressure from −0.8 to −2.8 cm. of water. The negative thoracic pressure in this case was able to exert a demonstrable "sucking" effect on the lymph vessels of the neck, thus producing a negative lymph pressure.

Slight oscillations in pressure, usually less than 1 mm. during quiet breathing and synchronous with respiratory movements, were observed in each animal. These oscillations were caused, as the earlier workers stated, by respiratory changes in intrathoracic pressure.

The effect of venous congestion and local increase in temperature on cervical lymph pressure. Landis and Gibbon (1933), using the human arm, have shown that an increase in venous pressure causes an accumulation of tissue fluid, the latter being due to an increase in the rate of blood capillary filtration. The rate of filtration was found to be directly proportional

TABLE 1

Cervical lymph pressures measured in centimeters of water during passive motion of the head of the dog

EXPERIMENT NO.	RANGE	AVERAGE	REMARKS
1	+0.4 to −0.2	+0.1	Respiration moderate
2	−0.8 to −2.8	−2.0	Respiration deep and fast
3	+1.5 to +1.3	+1.4	Respiration moderate
4	+1.0 to +0.2	+0.7	Respiration moderate
5	+1.0 to +0.6	+0.8	Respiration moderate
6	+3.2 to +2.8	+3.0	Respiration moderate

to the increase in venous pressure, provided the latter was above an average value of 12 cm. of water. Warming the arm to 44 to 45°C. during the period of venous congestion caused a decidedly greater increase in the rate of filtration from the blood capillaries as compared with that obtained during venous congestion alone. An increase in lymph flow results from the accumulation of tissue fluid, the amount of lymph varying according to factors such as the amount of filtration, available tissue space, tissue turgidity, motion of the part, etc.

The typical effects on cervical lymph flow of clamping the external jugular veins for 20 minutes with and without the simultaneous irrigation of the nasopharynx with saline at 45°C. are shown in figure 1. The increase in lymph flow at *A* with venous congestion produced during the application of local heat was almost twice that obtained at *B* with venous congestion alone.

The effects on cervical lymph pressure were not so marked. Venous congestion alone caused slight increases in two animals and slight decreases

in pressure in three animals. Figure 1 was taken from an experiment in which an increase in lymph pressure occurred when venous congestion for 20 minutes produced jugular vein pressures of 18.4 and 18.8 cm. of water. A rise of 0.4 cm. of water was caused by clamping the external jugular veins at *B*. This increase in pressure appeared before the increase in lymph flow was evident and was not maintained throughout the period of congestion. At *A* a rise of 0.5 cm. reaching its maximum in 10 minutes occurred when venous congestion was caused during the local application of heat. In this case it is evident that increases in capillary filtration

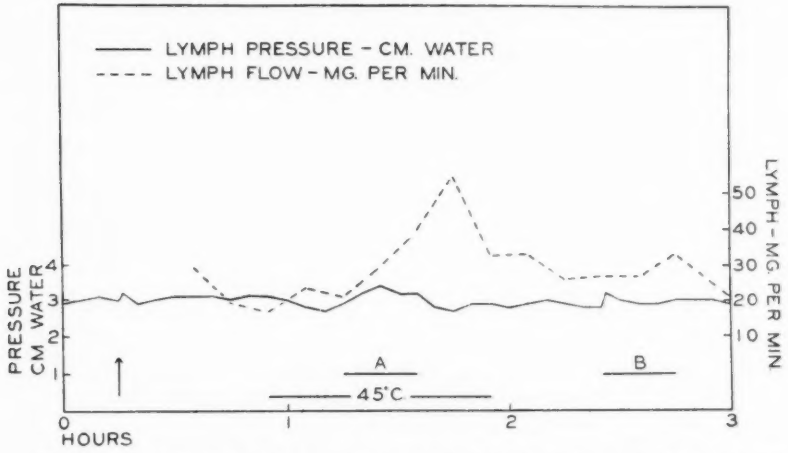

Fig. 1. The effect of venous congestion and of local application of heat to the nasopharynx on lymph pressure (right cervical lymphatic) and on lymph flow (left cervical lymphatic). Passive motion of the head of the dog begun at point marked by arrow and continued throughout the experiment; external jugular veins clamped at *A* for 20 minutes during irrigation of the nasopharynx with saline at 45°C.; a similar period of venous congestion alone at *B*. The external jugular vein pressure reached 18.8 cm. of water during *A* and 18.4 cm. of water during *B*. Note the marked increases in lymph flow and the very slight increases in lymph pressure.

rate capable of causing significant increases in lymph flow have only a slight effect on cervical lymph pressure.

An example of the interesting fall in lymph pressure associated with venous congestion which occurred in three animals is shown in figure 2 (animal 2). This illustrates an extreme case in which the pressure fell 8 mm. and remained at a low level as long as the jugular veins were clamped. Lymph flow measured from the same vessel during a similar period of venous congestion reached a particularly high level.

The fall in pressure which occurred during venous congestion is rather difficult to explain. One theory however is presented. The negative

thoracic pressure transmitted to the veins at the base of the neck exerts a slight aspirating effect on the blood and lymph vessels entering those veins. This mechanism was particularly effective in animal 2 in which it produced a negative cervical lymph pressure. When the external jugulars are clamped they collapse below the ligature and the aspirating effect is exerted more strongly on those vessels through which flow is unimpeded. This results in a decrease in cervical lymph pressure. In those cases in which an increase of lymph pressure occurred, the venous aspirating mechanism must have been negligible.

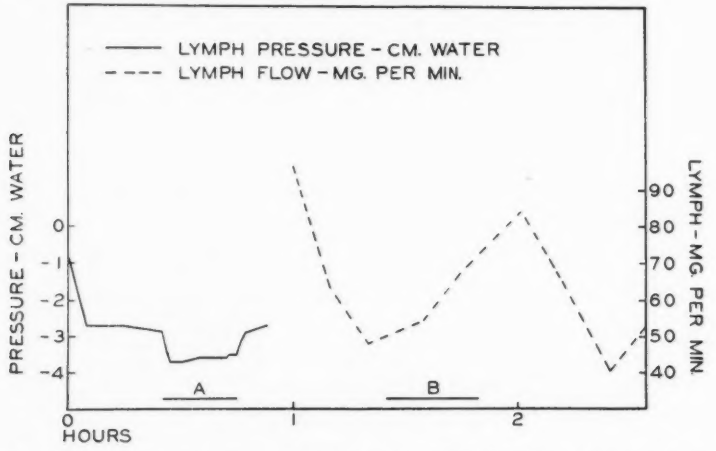

Fig. 2. Negative lymph pressure recorded from right cervical lymphatic in one dog. At *A* the external jugular veins were occluded for 20 minutes. The manometer was then disconnected, the lymphatic occluded and lymph flow determined from the same vessel. A second period of venous congestion at *B* produced a marked increase in lymph flow.

The external jugular vein pressures during venous congestion ranged from 12.7 to 14.3 cm. of water when a drop in lymph pressure occurred, and from 18.2 to 19.3 cm. of water when lymph pressure increased. The higher venous pressure in the latter instance no doubt contributed to the increase in lymph pressure. The actual venous pressures obtained by blockage of both external jugular veins is hard to control, as the degree of venous congestion depends largely on the efficiency of collateral veins draining the head and neck. In the present experiments complete venous stasis was not attempted.

End pressure. In two animals, both cervical lymphatics were obstructed below the cannulae and end pressures determined. In one case the pressure increased gradually over a period of four hours from −1.9

to +44.5 cm. of water. Clamping the external jugular veins caused no further increase. This pressure was maintained for a period of 40 minutes and dropped immediately to +0.5 cm. of water when the lymphatic was released. In a second animal, during a period of 2 hours and 45 minutes, the pressure rose slowly until it reached 21 cm. of water, where it remained stationary for 25 minutes. An intravenous injection of saline caused only a slight increase in the rate with which the pressure rose. Drinker and Field (1933) report pressures of 56 to 99 cm. of water in dogs when flow was obstructed in leg lymphatics just below the popliteal node and the foot was attached to a rotator. Lymphatics can be subjected to even higher pressures without being damaged seriously. Field, White and Drinker (1932) obtained pressures as high as 120 cm. of water in ankle lymphatics of the dog when a sterile inflammatory reaction was produced in the foot. It is evident from these experiments that the maximum pressures obtainable with lymphatic obstruction may vary considerably. Capillary filtration, area and elasticity of the lymphatic network, tissue turgidity, valvular efficiency, and motion of the part are some of the factors influencing the ultimate end pressures recorded in collecting lymphatics.

SUMMARY

A method, involving passive motion of the head previously described for the maintenance of a constant cervical lymph flow in anesthetized dogs, has been utilized for a study of cervical lymph pressure. Lateral lymph pressures, obtained by placing a T-cannula in one cervical lymph vessel, ranged from −2.8 to +3.2 cm. of water, with the majority of the pressures falling between +0.1 and +1.4 cm.

Venous congestion, sufficient to produce an increased lymph flow, in two cases caused a slight increase in cervical lymph pressure and in three cases a slight decrease, the latter being most evident in one instance in which the negative thoracic pressure produced a demonstrable aspirating effect on the cervical lymphatics. Venous congestion during the local application of heat to the nasopharynx produced a more striking increase in cervical lymph flow than in cervical lymph pressure.

End pressures, obtained by obstructing lymph flow, reached maximal values of 21.0 and 44.5 cm. of water. Normal cervical lymph pressures, in the absence of lymphatic obstruction, probably never reach values above a few centimeters of water. Factors such as the negative thoracic pressure, size and elasticity of the lymphatic network, efficiency of the valves, and motion of the part tend to keep the pressure at a minimum.

Dr. Cecil K. Drinker suggested this problem and gave valuable assistance and criticisms during its course.

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THE MAGNITUDE, ADEQUACY AND SOURCE OF THE COLLATERAL BLOOD FLOW AND PRESSURE IN CHRONICALLY OCCLUDED CORONARY ARTERIES[1]

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Although Mautz and Gregg (1) found that following chronic obstruction of a coronary artery a collateral circulation is at times spontaneously established (four of five experiments), we have no knowledge of 1, the volume and character of the collateral flow; 2, the source of this blood flow; 3, the pressure head under which it enters the peripheral end of the centrally occluded coronary artery, and 4, the extent to which it can replace the normal blood supply of the occluded coronary artery. This latter point is of added significance in view of the contention (Beck, 2) that autogenous grafts surgically applied to the myocardium may serve as a vascular bridge from one portion of the heart to another. Accordingly, methods of study have been devised in an attempt to obtain the answers to these questions on a quantitative basis.

Surgical preparation. Chronic coronary arterial occlusions in dogs lasting from thirty-six to three hundred sixty-five days were produced in three ways: 1, a major coronary artery was dissected free as close to its ostium as possible and divided between ligatures; 2, a screw clamp (3), designed to produce gradual occlusion, was applied to a single main coronary artery at its origin and in three to six subsequent operations on the same dog the clamp was tightened until the arterial lumen was completely closed, and 3, either the magna corda vein or the coronary sinus was ligated and thirty days later a coronary artery was ligated as in the first method. Twenty-three dogs survived sufficiently long after such complete occlusion of a coronary artery so that the compensatory changes in coronary hemodynamics could be successfully investigated.

[1] This investigation was aided by grants from the American Medical Association to one of us (Gregg) and from the Josiah Macy, Jr. Foundation to the Department of Surgery. Preliminary reports of this work were given before the joint meeting of the Experimental Medicine Section of the Cleveland Academy of Medicine and the Cleveland Section of the Society for Experimental Biology and Medicine on January 13, 1939 and before the American Physiological Society, Toronto, Canada, April 27, 1939.

EXPERIMENTAL PROCEDURE. When sufficient time had elapsed for the development of a collateral circulation, the dogs were sacrificed in acute experiments. They were anesthetized either with morphine and ether or by intravenous use of sodium barbital, amytal or pentobarbital. The heart was exposed and the coronary arteries to be studied were dissected free. The blood was rendered non-coagulable by heparin and chlorazol fast pink or by heparin alone. Aortic pressure and peripheral coronary pressure just below the point of occlusion were recorded optically by the Gregg type manometer (4, 5). In most experiments the retrograde flow from an opened coronary vessel was measured. In the earlier experiments this was collected in a graduated cylinder, or registered by an optically recording Archimedes bucket. In the later experiments it was collected in a bottle of suitable size in which the pressure did not exceed 1 mm. Hg. The bottle was connected by rubber tubing to a large segment capsule which recorded optically. During the course of the experiments systemic blood pressure was controlled either by an adjustable clamp around the aorta or by the use of a compensator similar to that described by Greene (6). Blood volume and venous return were maintained by infusion of Locke's solution or blood at body temperature.

The retrograde coronary blood flow and peripheral pressure. When a coronary artery of a normal dog is ligated and cut, the blood flow from its peripheral end never exceeds 1 to 1.5 cc. per minute and it is generally very much less. By comparison the peripheral flows from our operated dogs are quite large (compare typical experiments in table 1). In the left coronary rami large increases in flow have never failed to be established; in the descendens these have varied from 6 to 105 cc. per minute, in the circumflex from 32 to 84 cc. per minute. In the right coronary artery the peripheral flows are generally less than in the left (at the same aortic blood pressure) and have varied from 0.1 to 80 cc. per minute.

The systolic and diastolic pressures in the peripheral ends of chronically occluded coronary vessels were similarly affected. In normal dogs the order of magnitude is typified by the data included in table 1, viz., ramus descendens 36/22 mm. Hg, left circumflex 29/18 mm. Hg, right coronary 30/24 mm. Hg. After permanent occlusion the systolic and diastolic pressures were much higher (especially the systolic) with the exception of experiment 16 (see also fig. 1, H) in which the flow also failed to increase and presumably collateral circuits had failed to develop. The average peripheral pressures, including many experiments not in table 1, are descendens 82/38, circumflex 72/38 and right coronary 68/51 mm. Hg.

As expected, acute alterations in coronary hemodynamics, such as increased venous return or elevation of blood pressure, result in corresponding changes in peripheral coronary pressure and flow. For example, in table 1, experiment 18, a, b, c, as the aortic pressure gradually rises through

mild constriction of the aorta from 93/70 mm. Hg to 118/92 mm. Hg, the retrograde flow increases from 41 to 60 cc. per minute and the peripheral coronary pressure from 75/60 mm. Hg to 98/75 mm. Hg. The same trend is found later in the same experiment (18, d, e) when 200 cc. of Locke's solution warmed to body temperature were infused.

TABLE 1

EXPERIMENT NUMBER	NUMBER OPERATIONS	DURATION OF COMPLETE OCCLUSION	WEIGHT OF HEART	AORTIC PRESSURE	PERIPHERAL CORONARY PRESSURE	CORONARY FLOW
			Descendens			
		days	*grams*			*cc./mm.*
Control	0	0		88/52	36/22	<1.0
2	3	50		125/92	118/76	50.0
5	4	106	140	78/53	63/28	21.0
				117/68	85/24	34.0
1	1	60	72	82/54	67/40	14.0
Average of all experiments				102/66	82/38	25.0
			Circumflex			
Control	0	0		110/88	29/18	<1.0
8	4	129	138	111/47	69/31	84.0
9	5	106	142	95/60	55/25	32.0
				110/78	82/47	74.0
13*	2	46	128	104/54	77/44	60.0
Average of all experiments				107/60	72/38	58.0
			Right			
Control	0	0		140/97	30/24	<1.0
21	4	98	193	94/70	43/38	5.9
				102/70	48/37	12.7
18a	1	360	181	93/70	75/60	41.0
18b				105/80	91/74	52.0
18c				118/92	98/75	60.0
18d				125/88	100/75	65.0
18e				132/104	110/92	80.0
16	1	240	73	80/50	11/10	0.1
Average of all experiments				102/71	68/51	27.0

* Coronary sinus also chronically ligated.

The experimental findings, after permanent occlusion of any of the major coronary rami, of massive increases in the peripheral coronary pressure values and retrograde flows so that at times the former approaches the prevailing aortic pressure and the latter the normal volume flow that might be expected to enter the artery centrally were it not occluded, leave

no room for doubt that a considerable collateral circulation develops following chronic occlusion of coronary arteries.

Contour of peripheral coronary pressure curves. Previous studies (7, 8) on normal dogs have shown that the pressure curves obtained from the peripheral ends of the major coronary rami are fairly constant as regards form, magnitude and time relations with the exception of the right coronary in which the magnitude is less. This was again confirmed in this investigation.

A careful study of a large number of curves from dogs with long continued occlusion of coronary arteries showed that the pressure curves in their peripheral ends are generally altered but not according to any consistent pattern. It was also clear that no constant differences existed between the peripheral pulse patterns of the right or left coronary arteries. In figure 1 are presented normal curves from the peripheral ends of the ramus descendens (A), circumflex (D) and right coronary (G). For comparison with each the curves from corresponding vessels after long continued occlusion are shown in B, C; E, F; and H, I, J respectively.

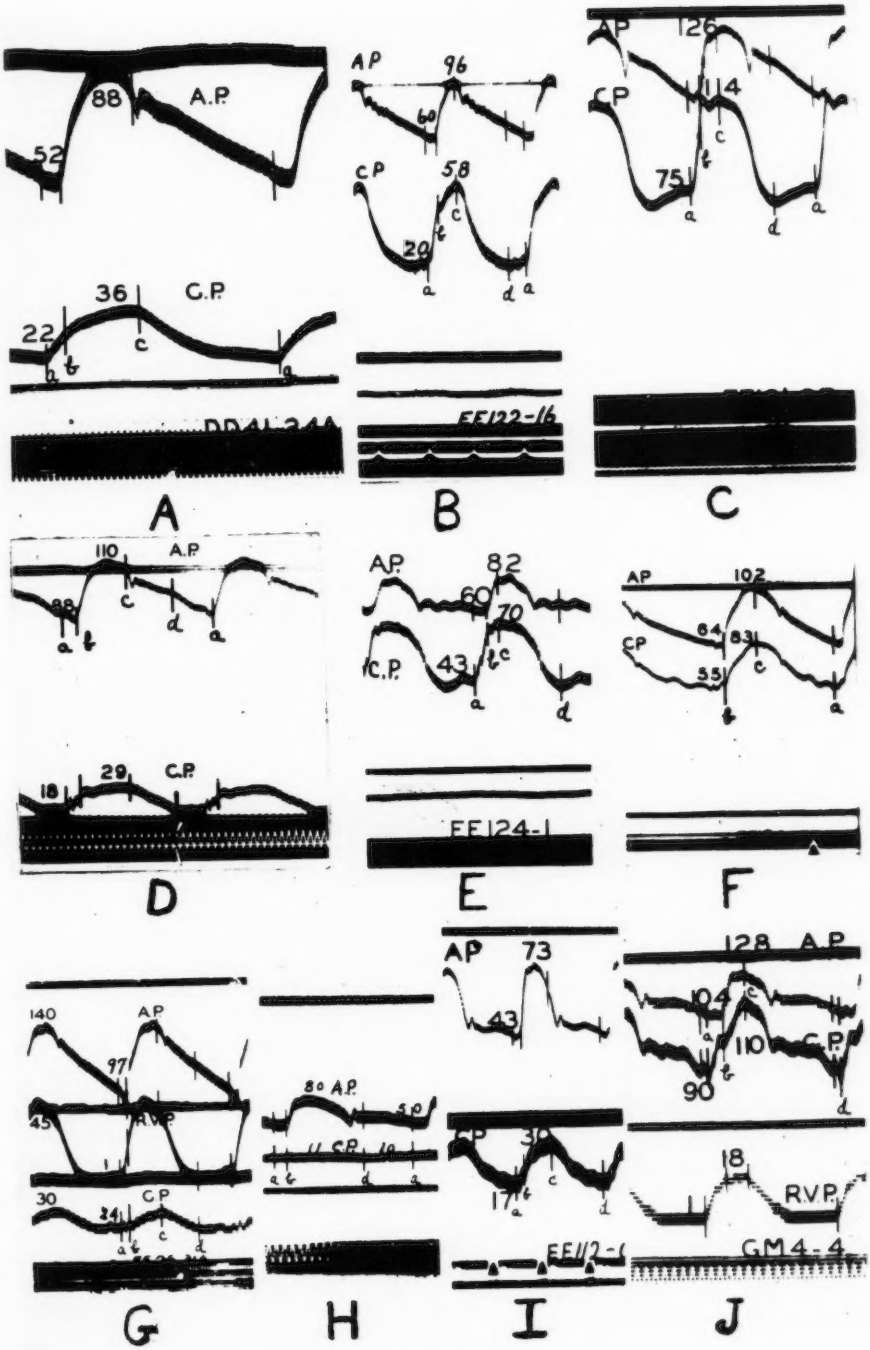
The obvious differences are: 1, the diastolic pressure which normally declines or remains constant during the latter part of diastole may be replaced by a rise (C, E); 2, the summit which normally occurs late in systole can come much earlier (C, E, J) or it may remain normal (B, I); 3, the contours of the aortic and peripheral pressure curves are at times strikingly similar (F, J).

Of these pulses the most usual pattern is that which is very similar to the normal peripheral coronary pulse; the types contained in C, E occur less frequently, while in only two experiments have we found coronary pressure curves scarcely distinguishable from the aortic.

Character of retrograde coronary blood. Although the preceding results demonstrate conclusively that good collateral beds develop spontaneously following chronic coronary occlusion, the deduction cannot be made that such a large volume flow under a good head of pressure is sufficient to maintain the metabolic requirements of the potentially infarcted area. It can well be argued that unless the blood is arterial in nature the minute volume of oxygen supplied will be insufficient to maintain contractions.

Accordingly oxygen and carbon dioxide determinations were made on samples of retrograde blood collected under oil and flowing from each of the three major coronary rami chronically occluded in different experi-

Fig. 1. Records illustrating the effects of long continued coronary artery occlusion on the peripheral coronary pressure. A, D, G, normal peripheral pressures in descendens, circumflex and right coronaries respectively. Other curves after persistent coronary occlusion, descendens, B, C; circumflex, E, F; right, H, I, J. *A. P.*, aortic pressure. *C. P.*, peripheral coronary pressure. *a, b, c, d*, vertical intercepts to facilitate curve reading. Time 0.02 sec. (A, C, D, G, H, J), 0.2 sec. (I).


88
A.P.
52
A.P
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126
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36
C.P.
22
A
B
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110
A.P.
82
60
70
C.P.
43
102
64
83
55
29
C.P.
18
D
E
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140
A.P
97
45
R.V.P.
30
C.P
34
80 A.P.
50
11 C.P.
10
73
43
128
A.P
104
110
C.P.
90
18
R.V.P.
G
H
I
J

Fig. 1

ments. From typical analyses contained in table 2 it is apparent that the collateral blood in all of the coronaries is arterial in nature. Although gas analyses were not made in all experiments, we feel that this blood is always arterial, for visual inspection of it always showed it to be of a bright red color. Such results and observations leave no room for doubt that the collateral circulation established is on the arterial side of the coronary capillary bed.

Ability of collateral circulation to maintain myocardial contractions. Although such findings definitely indicate that a new arterial blood supply of good volume and under a good pressure head does develop spontaneously in a potentially infarcted area it gives no information as to whether it is sufficient in quantity to nourish the muscle mass previously rendered ischemic. Indeed, it is highly improbable that flows of such magnitude as were obtained experimentally against atmospheric pressure exist when the chronically occluded coronary is not permitted to bleed. In our series

TABLE 2*

EXPERIMENT NUMBER	ARTERY CHRONICALLY OCCLUDED	RETROGRADE CORONARY FLOW			FEMORAL ARTERY, VOLUME PER CENT		VOLUME PER CENT O_2		O_2 CAPACITY
		cc. per min.	volume per cent O_2	volume per cent CO_2	O_2	CO_2	L. vent	Rt. vent	
2	Descendens	50	13.08	30.82	13.13	30.00		8.36	15.79
19	Right	20	15.56				15.53	8.38	20.24
13	Circumflex	60	11.1		11.6				13.08

* For these analyses we are indebted to Dr. Edward Muntwyler of the Department of Biochemistry, Western Reserve University.

in which a coronary was occluded in one stage the myocardium fed by it immediately became cyanotic and expanded with each heart beat rather than shortened. This agrees with hundreds of other experiments in which, after a coronary artery had been occluded for various reasons, the ischemic myocardium failed to contract in from one to two minutes. Therefore, the conclusion seems justified that any persistence or recurrence of contraction in hearts with complete coronary occlusion is caused by the appearance of newly functioning collaterals. In all the experiments presented here the heart beating in situ was carefully examined for evidence of gross scarring or failure of myocardial systolic shortening in the region normally fed by the chronically occluded coronary. In most (but not all) hearts small areas of scarring were present, and in one experiment (16, table 1, and also fig. 1, H) with right coronary occlusion the entire area normally supplied by the right coronary artery was involved and with no evidence of contraction. In all other animals, however, the potentially infarcted area was visibly shortening during systole. A typical myogram (9)

taken from the myocardial region of the left descendens after 106 days of occlusion is contained in figure 2. The record shows systolic shortening (a–b).

The source of the newly established collateral circulation. Anatomical considerations suggest several source possibilities: 1, the large intercoronary communications found following chronic occlusion and previously described (1); 2, anastomoses between the coronary arterial tree and extracoronary arteries reaching the heart along the course of the great vessels (10), and 3, anatomical pathways from the cavities of the heart to the centrally occluded segment by the channels described by Wearn (11). Of these possibilities, only one, the possible rôle that the intercoronary communications might play in the collateral circulation, has been subjected to direct experimental investigation.

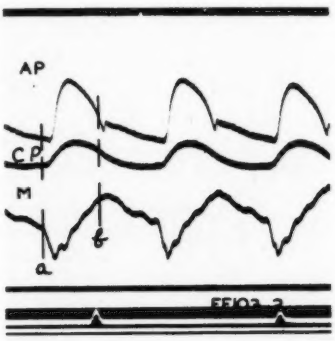

Fig. 2. Record showing systolic shortening in myocardial area of a chronically occluded ramus descendens. *M*, myogram. Vertical intercepts, duration of systole. Time 1.0 sec. Other letters same as figure 1.

The method used to obtain this information consisted in clamping the other two coronaries separately while the peripheral pressure and flow were being recorded in the coronary chronically occluded. The immediate and stabilized changes produced in different coronary arteries are illustrated in detail in the curves of figures 3 and 4.

In the curves of figure 3 the ramus descendens had been clamped for 50 days. The first two beats in segment *A* represent the normal aortic pressure, 113/86 mm. Hg, and the pressure, 110/61 mm. Hg, in the peripheral end of the chronically occluded descendens. The normal retrograde flow was 52 cc. per minute for a one minute period. At time *X* the left circumflex is suddenly occluded and the peripheral pressure decrease within three heart beats to 56/23 mm. Hg. After 15 heart beats (only the last beat shown here) the peripheral coronary pressure attains a new

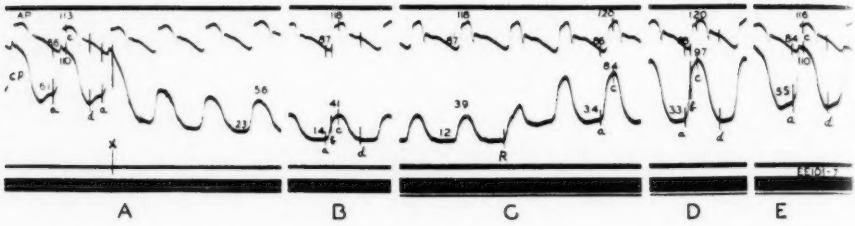

Fig. 3. Sections from a continuous record illustrating the immediate effects of left circumflex occlusion on peripheral coronary pressure in chronically occluded ramus descendens anterior. A, normal control with circumflex ligation at *X*. B, same but 18 heart beats later. C, Circumflex release at R. D and E, taken 30 and 60 seconds later. Other letters same as figure 1. Time 0.02 sec.

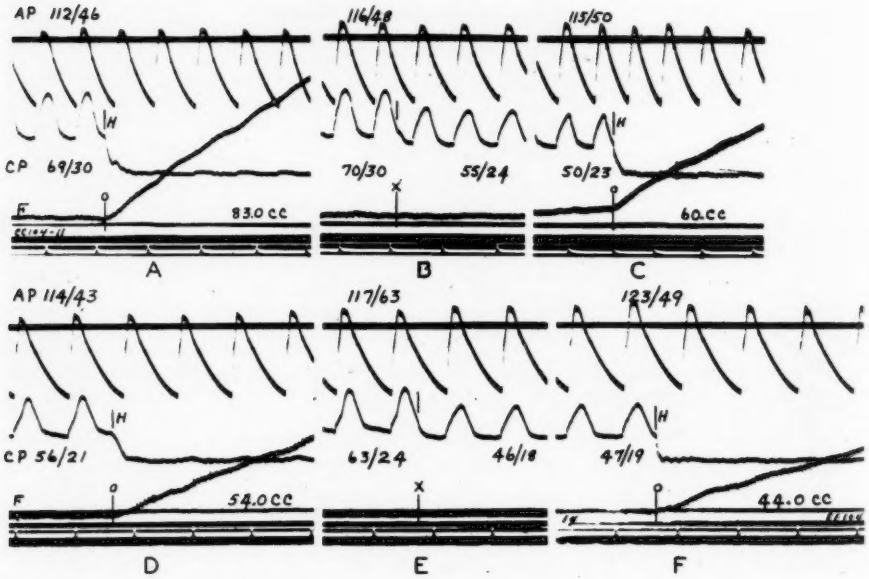

Fig. 4. Reproductions of segments of continuous records showing effect of separate and acute ligation of the right and descendens on the retrograde flow and pressure in the chronically occluded circumflex. A, control flow and pressure for right coronary ligation in B, C. D, control pressure and flow prior to descendens occlusion in E, F. *H*, time at which back flow started. *O*, beginning of flow recording. *X*, time of coronary ligation. F, flow curve. Other letters same as figure 1. Time 0.2 sec.

and final equilibrium of 41/14 mm. Hg and the backflow is 10 cc. per minute. Approximately three heart beats later in segment C the left circumflex was released at R and the peripheral pressure immediately rises to

84/34 mm. Hg. in C. In E, recorded 60 seconds later (30 seconds after D) the peripheral coronary pressure of 110/55 mm. Hg is back to the control figures in segment A while the back flow at this time is 53 cc. per minute.

While the rôle of the right coronary as a collateral source for the descendens was not determined in this experiment, it is obvious that its maximum contribution together with all remaining collaterals is 19 per cent. In five dogs the contribution of the circumflex in back flow has varied from 37 to 81 per cent and in peripheral pressure from 65/76 to 32/39 per cent. In other experiments on the chronic descendens the maximum reductions in peripheral pressure and flow after clamping the right coronary have been 5/18 and 14 per cent respectively.

Following sudden artery occlusion in A at point *X* the systolic portion of the coronary curve shifts from a spike type early in systole to one which is well rounded and which tends to reach its peak rather late in systole at *c* as the occlusion is continued through segments B and C. In the normal segment A before left circumflex occlusion the diastolic portion of the coronary curve rises rather rapidly during the latter half of diastole. Following artery occlusion at X and continued through segments B and C the same diastolic portion of the coronary curve falls rather than rises during this time interval. Upon artery release in segment C at point R the curve gradually resumes its original form (segments C, D, E).

The separate contributions in pressure and flow of the ramus descendens and right coronary to the chronically occluded circumflex are presented in figure 4. Following descendens ligation in B the retrograde flow and pressure drop from control figures of 83 cc. per minute and 69/30 mm. Hg in A to 60 cc. per minute and 50/23 mm. Hg in B. In six determinations in different experiments the flow reductions have ranged from 24 to 64 per cent while the peripheral pressure reductions have varied from 22/21 to 44/51 per cent. In the lower records of figure 4 the right coronary was suddenly occluded in E and the peripheral pressure and flow decrease from control values in D of 56/21 mm. Hg and 54 cc. per minute to 47/19 mm. Hg and 44 cc. per minute in E. The range of such pressure and flow reductions in six experiments has been from 8 to 27 per cent for flow and 5/0 to 22/22 per cent for pressure.

After chronic occlusion of the right coronary the sudden clamping of the descendens gives but a very small reduction in peripheral pressure and flow, but circumflex occlusion results in large pressure and flow reductions (record not shown here because of space limitations).

This large change in form and reduction in magnitude of the peripheral pressure and retrograde flow in a chronically occluded coronary are representative of what happens in any chronically occluded cornary when another coronary which has established new collateral communications with it is suddenly ligated.

The residual peripheral coronary pressure and flow after occlusion of all

coronaries. When a coronary artery has been chronically occluded and both of the other major coronary rami are clamped separately, the sum of the flow reductions may or may not approximate 100 per cent. This depends upon which coronary has been chronically occluded. In the circumflex or the descendens the flow reductions by clamping the other two coronaries do not approximate 100 per cent, whereas in the right they generally do. All our data on this point are briefly summarized in table 3. It can be seen that in the right coronary chronically occluded essentially all the retrograde flow comes from the other coronaries (most from the circumflex), while in the descendens only 62 per cent comes from the right (7 per cent) and circumflex (55 per cent) and in the circumflex only 66

TABLE 3

ARTERY CHRONICALLY OCCLUDED	ARTERY ACUTELY OCCLUDED	PER CENT REDUCTION PERIPH. CORONARY, SINGLE		PER CENT REDUCTION PERIPH. CORONARY, COMBINED		PER CENT POTENTIAL EXTRACORONARY COLLATERAL FLOW
		Flow	Press.	Flow	Press.	
Descendens	Right	7	3/10	62	55/74	38
	Left circumflex	55	52/64			
	*Right and left circumflex			66	49/50	34
Left circumflex	Right	19	15/11	66	48/48	34
	Descendens	47	33/37			
	*Right and descendens			64	59/54	36
Right	Descendens	22	10/11	97	71/72	3
	Circumflex	75	61/61			
	*Descendens and circumflex			79	59/57	21

* Arteries simultaneously occluded.

per cent comes from the other coronaries (right 19 and descendens 47 per cent).

This finding, that apparently a portion of the collateral circulation developed in the vascular bed of either left coronary ramus (but not the right) following long continued occlusion has an extracoronary source, is open to several interpretations: 1, the residual flow may come from a coronary branch central to the clamp; 2, acute occlusion of one coronary which has developed sizeable anastomoses with a chronically occluded coronary might augment the retrograde flow arising in a third unoccluded coronary and thus minimize the flow figure from the first coronary, and 3, the residual flow has a true extracoronary origin.

To obtain information regarding these possibilities, the effects of simul-

taneous closure of the remaining coronaries on the peripheral pressure and flow in the third coronary were recorded. The hearts (12 experiments) withstood the strain quite well. The results are in table 3 and figure 5.

When, after the right coronary has been occluded for 98 days, the common left is abruptly ligated, the peripheral pressure and retrograde flow decrease from 43/33 mm. Hg and 10.7 cc. per minute (fig. 5, C) to 19/5 mm. Hg and 1.5 cc. per minute (fig. 5, D). If it is assumed that the normal backflow in the right coronary is about 1 cc. per minute, then 94 per

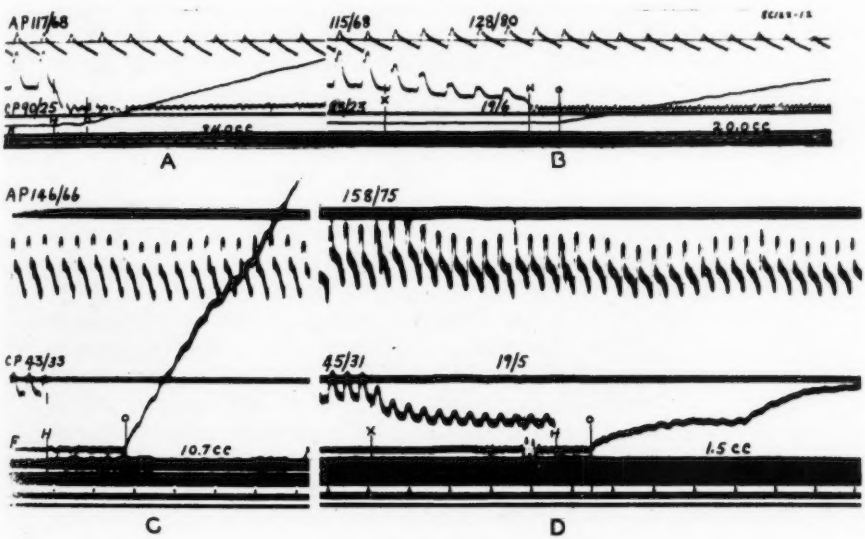

Fig. 5. Records exhibiting the effect of simultaneous acute ligation of two coronary arteries on the peripheral pressure and blood flow in the third coronary chronically occluded. A, chronic descendens as control. B, one minute later with right and circumflex occluded. C, chronic right as control. D, one minute later with common left ligated. Letters and time interval same as figure 4.

cent of the retrograde flow in this experiment comes from the common left coronary. In four animals the common left has never accounted for less than 85 per cent of the right coronary retrograde flow.

If the descendens has been ligated for 106 days and then the right and circumflex are occluded simultaneously, the peripheral coronary pressure drops from 90/25 to 19/6 mm. Hg and the backflow from 34 to 20 cc. per minute (fig. 5 A vs B), or 59 per cent of the collateral flow has an extracoronary origin. Similar reductions in peripheral flow and pressure have been found in the chronically occluded circumflex and the results for all

three arteries, i.e., chronically occluded and in which the other two have been simultaneously ligated in sacrifice experiments, are summarized in table 3. Comparison of these results with those found after separate ligation of the coronary arteries (also table 3) shows that the potential extracoronary flow is of the same order of magnitude in the three arteries by either method.

Discussion. The experimental findings in 22 of 23 dogs, following chronic removal of the normal blood supply by mechanical occlusion of any of the major coronary rami, of 1, large augmentation in retrograde blood flow (arterial); 2, large increases in peripheral coronary pressure over normal values; 3, minimal scarring; 4, myocardial contractions (by visual observations and myographic records) give weighty evidence that a collateral circulation of substantial magnitude has been established.

However, there is a considerable natural variation in the ability of such anastomotic circuits to develop in different animals. In our hands, as well as in the hands of others, the mortality accompanying these single or multiple operations is quite high. Those dogs which survived complete occlusion of a coronary without doubt represent a select group naturally endowed with a high resistance toward this type of procedure. While it is not known why some dogs survive and others do not, it is certain that those dogs reported here do not represent a group in which large collateral connections already existed before coronary occlusion, since in a large number of experiments in which the backflows and peripheral coronary pressures have been measured immediately after coronary artery occlusion, no flows have been greater than 1.5 cc. per minute, the peripheral coronary pressures have been quite low and the myocardium has never persisted in contracting.

Associated with the augmented retrograde flow following chronic occlusion there is a marked elevation of the peripheral coronary pressure (table 1, fig. 1). Since this rises and falls in any one experiment with concordant changes in retrograde flow, and its contour is limited to definite patterns, examination of them should reveal in part at least the source and time of entry of blood and pressure into the occluded coronary.

In two experiments (fig. 1, F, J) it is quite certain that most of the peripheral pressure head and therefore retrograde blood comes from an artery near the aortic valves, for the systolic and diastolic values of the peripheral pulse approach the aortic pressure simultaneously recorded, most of the rise occurs after opening of the aortic valves and even an incisura is present; or, in other words, the peripheral coronary curve parallels in all respects the aortic. It is, therefore, logical to assume that the connections between this artery or arteries and the vascular bed of the occluded coronary are of good cross section and superficially located in the myocardium. Actually this was found to be the case, for röntgenograms of one of these

injected hearts (J) showed a large vessel connecting the occluded artery with the circumflex.

This transition from the normal peripheral pulse representative of that artery (fig. 1, D, G) to one resembling the aortic or central coronary pressure pulse must mean that the pulse is no longer a partial measure of the intracoronary resistance but rather represents the head of pressure entering the area from some artery, either coronary or extracoronary. Viewed in this light, namely, that the peripheral coronary pressure has now become a central one, and since we have no reason to suppose that the peripheral coronary resistance has materially changed from the normal (unpublished experiments), it would be predicted that the phasic blood flow in it would be similar to that occurring in the unobstructed left and right coronaries, that is, a greater volume flow in the left during diastole (12) and in the right a greater systolic flow for the same time interval (8).

Of the remaining coronary pulses some are similar to the peripheral coronary pressures obtained immediately after coronary ligation, others more nearly resemble the ventricular pulse, while none are similar to a known arterial pulse (except for their high systolic and diastolic values). Since the source is known to be largely some artery, as demonstrated by clamping the other coronaries, the question arises why so few of the curves resemble an arterial pulse. It is quite likely that the differences in configuration of the peripheral coronary pulses are caused by the fact that in most the collateral architecture and location are such as to distort the curve, i.e., they consist of many small superficial connections or they are of small or large size but deeply buried in the myocardium. This concept has been borne out by injection studies.

From an examination of such pressure curves found in chronically occluded coronaries it is believed that most of the flow through the vascular bed of the occluded coronary is at a minimum during systole, for most of the rise of the peripheral pressure precedes the opening of the aortic valves; and is at a maximum during diastole, for the diastolic pressure after isometric relaxation (when the capacity of the vascular bed is presumably constant) is either rising or falling. This view is substantiated by optical records of the phasic blood flow into the occluded coronary (flow meter and results to be described later).

The interesting fact is brought out that, although by direct determination the augmented retrograde flow and peripheral coronary pressure in a chronically occluded coronary have their major origin in the non-occluded coronaries, there remains a large fraction of the flow which cannot be removed by clamping the other coronaries (table 3). This potential extracoronary retrograde flow has been quite small in the right coronary but large in the descendens and circumflex. However, residual flows of good volume are not always present in the chronic descendens or circumflex

with the other two coronaries acutely closed, while at times in the right the reverse is true.

Although such residual flows measured against atmospheric pressure are without doubt somewhat exaggerated, since in the closed vessel the resistance to flow is much greater, we have no reason to doubt its existence in many of our experiments. The hearts stood up remarkably well under this procedure maintaining a fairly good blood pressure for several minutes. However, as to be expected in those experiments in which the coronaries remained clamped, within a short time the blood pressure dropped and the heart fibrillated, presumably largely as the result of the altered dynamics in the acutely occluded coronaries.

CONCLUSIONS

The results presented elucidate in part what happens dynamically in the vascular bed of a chronically occluded coronary artery in favorable dogs and have led to the belief that a large new collateral circulation develops. Our criteria for such a belief are the recorded experimental facts that 1, in 22 of 23 dogs the peripheral coronary pressure is greatly increased (as compared to normal controls) and at times to values even approaching aortic pressures; 2, the retrograde blood coming from the peripheral end of the occluded coronary ranges up to 105 cc. per minute with most of the values around 30 to 40 cc. per minute; 3, the retrograde blood is so similar to arterial blood that it cannot be differentiated on the basis of its carbon dioxide and oxygen content, and 4, such volume flows of arterial blood are sufficient for the metabolic needs of the potentially infarcted myocardium, for the myocardial region exhibits normal contractions except in areas of scarring.

No single pulse pattern or set of ordinate values exists in the peripheral ends of the major coronary rami chronically ligated. The most usual peripheral coronary pressure is similar in timing and contour (although greater in magnitude) to the normal curve obtained immediately after coronary ligation, others more nearly resemble an intraventricular pressure curve while less frequently the peripheral coronary pressure curve can scarcely be distinguished from the aortic curve simultaneously recorded.

From an analysis of these coronary pulse patterns the conclusion is drawn that most of the collateral flow occurs during diastole and that only in those coronary pressure curves which resemble the aortic in ordinate values and contour can the source of the retrograde blood be predicted.

By clamping the other coronaries either separately or together while measuring the retrograde flow in the third coronary chronically occluded, the functional extent of the newly established coronary anastomoses has been determined. It has been found that:

1. The descendens receives on an average 62 to 66 per cent of its collateral flow from the other coronaries (right 7 per cent and circumflex 55 per cent).

2. The circumflex receives 66 to 64 per cent from the other coronaries of which the right and descendens contribute 19 and 47 per cent.

3. The right receives 97 to 79 per cent of its flow from the left coronary artery, the descendens contributing 22 per cent and the circumflex 75 per cent.

This leaves a large residual flow in the left coronary and a small residual flow in the right still to be accounted for. The origin of this potential extracoronary retrograde flow has not been determined.

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THE LOCATION AND FUNCTION OF THE CHEMORECEPTORS OF THE AORTA[1]

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Although Heymans and Heymans (1927) demonstrated the presence of chemically sensitive nerve receptors in the region of the aortic arch several years before Heymans et al. (1931) discovered similar receptors in the carotid sinus area, the former have received little attention since that time while the latter have been investigated repeatedly. Consequently the localization and physiological significance of the carotid receptors are now fairly well understood, but even the existence of the aortic receptors has been denied (Dautrebande and Wegria, 1937; Beyne, Gautrelet and Halpern, 1933), and their localization and significance are both uncertain. A number of investigators (Selladurai and Wright, 1932; Schmidt, 1932; Jongbloed, 1936; Gesell and Moyer, 1937; Lambert and Gellhorn, 1938) have confirmed the presence of extracarotid chemoreceptors by showing that, even after complete carotid denervation, anoxemia still produces some increase in respiration which disappears when the vagodepressor nerves are cut. However no attempts at precise physiological localization have been reported since the work of Heymans and Heymans (1927). Anatomical studies made by Penitschka (1931), Palme (1934), Muratori (1934), Seto (1935), Nonidez (1935, 1937), and Boyd (1937) have demonstrated the presence about the aortic arch of cell groups which are similar in appearance to the chemoreceptors of the carotid bodies—a structural relationship which has led to the suggestion that these cells represent the chemically sensitive areas of the aortic region. Until now this suggestion lacked physiological confirmation.

The objects of the present experiments were to ascertain the anatomical

[1] A preliminary report of these experiments was made at the meeting of the American Physiological Society at Baltimore, Md., on March 31, 1938 (This Journal **123**: 41, 1938) and at the 16th International Physiological Congress at Zurich, August 17, 1938.

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and physiological characteristics of the aortic chemoreceptors, i.e., to determine their location, blood and nerve supply, and to obtain information about their significance to the organism. Owing to the relative inaccessibility of the aortic reflex zone, the various perfusion methods which have been successfully employed by a number of workers in investigating carotid reflexes, when applied to the aortic region, involve such wide departures from normal conditions that the results are of limited value beyond an indication that reflexes are aroused there by certain chemical agents. We hoped that useful information could be obtained by simple methods, now that the characteristics of the carotid reflex mechanism are fairly well understood; this hope was justified.

The experiments were performed on dogs and cats under anesthesia by chloralose (about 50 mgm. per kilogram) supplemented by urethane (about 0.6 gram per kilogram, this and the chloralose being given together intraperitoneally in dogs and cats) or by morphine (2 mgm. per kilo intramuscularly in dogs, following which the chloralose was given intravenously). A valved tracheal cannula was inserted; blood pressure was recorded by a mercury manometer from a femoral artery with thiosulfate or heparin-saline in the cannula; respiration was recorded by a pneumograph and tambour and occasionally the volume of expired air was measured by a gas meter. The sinus and aortic nerves and their receptor fields were scrupulously avoided until it was desired to inactivate them.

1. *The physiological significance of the aortic chemoreceptors in the dog.* Information on this point was obtained by testing the responses of respiration and blood pressure to systemic anoxemia (produced by inhalation of nitrous oxide) and to intravenous injections of α-lobeline and sodium cyanide, before and after complete bilateral carotid denervation. The responses after the denervation were assumed to be due to aortic reflexes — an assumption which appears to be justified, as will be seen below.

It should be emphasized at the outset that the results elicited by these simple procedures were quite variable from animal to animal, not only in the intensity of the total response (hyperpnea and hypertension) produced by the same agency in different animals, but also in the partition of the total response between the carotid and aortic receptors. However, three results were consistently obtained:

1. The hyperpneas produced by acute anoxemia and by α-lobeline and cyanide were usually reduced considerably though never completely abolished by carotid denervation.

2. The hypertension during acute anoxemia was usually intensified, and that produced by intravenous injections of α-lobeline and cyanide was always greatly intensified, after carotid denervation.

3. The hyperpnea and hypertension elicited by any of these agents after carotid denervation as a rule were abolished by section or blocking

of the depressor or vagodepressor nerves and pure depression of respiration and blood pressure occurred.[2]

The type of evidence upon which these statements are based is illustrated in figure 1. In the intact dog (fig. 1a) anoxemia caused intense hyperpnea and marked hypertension. When both vagi were blocked with procaine (fig. 1b) anoxemia still caused marked hyperpnea but the hypertension was completely lacking although the carotids were untouched. After the vagus block wore away (fig. 1c) anoxemia again produced both hypertension and hyperpnea. When both carotids were denervated with vagi intact (fig. 1d) the hyperpnea of anoxemia was greatly reduced though not abolished, while the hypertension was greater than before. Finally when both vagi were again blocked after carotid denervation, (fig. 1e), anoxemia failed to cause any increase in either blood pressure or respiration.

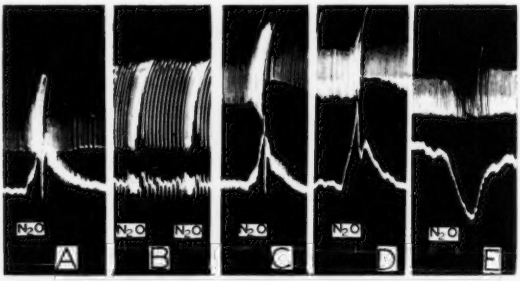

Fig. 1. Effects of systemic anoxia with all chemoreceptors intact (A and C), with only carotid chemoreceptors intact (B), with only aortic chemoreceptors intact (D), with all chemoreceptors denervated (E). See text. Dog. Morphine and chloralose. Pure nitrous oxide inhalation given between marks. From above downward: Respiration (pneumograph), femoral B.P., signal, time in 5 sec. intervals.

As pointed out above, the results of this simple experiment were quite variable from animal to animal. To show the nature and extent of these variations it will be necessary to take up the respiratory and circulatory effects separately. The *respiratory* effects shown in figure 1 are typical of

[2] There are several exceptions to this statement: (1) A smaller, more gradual increase in blood pressure followed intravenous injections of large doses (1.0 mgm.) of α lobeline, presumably due to direct sympathetic ganglion stimulation. (2) An increase in respiration sometimes occurred during systemic anoxemia even after denervation of the carotid and aortic chemoreceptors. Occasionally this occurred only after the N_2O inhalation was carried to the point of an abrupt fall in blood pressure, and was probably due to the accumulation of metabolic products in the respiratory center because of reduction in its blood supply (Gesell, 1925; Schmidt, 1928, 1932). In a few instances a slight, delayed increase in respiration occurred without fall in blood pressure. In the absence of other known peripheral chemoreceptors this probably represents a direct stimulation of the respiratory center as described by Gesell (1939).

those obtained in 12 out of 49 dogs (24 per cent) subjected to inhalation of N_2O or to intravenous injection of α-lobeline or cyanide, before and after carotid denervation. In such cases the hyperpnea of aortic origin (fig. 1d), while distinct, was decidedly less than that arising in the carotids. In another 18 of these 49 animals (37 per cent) the aortic component of the total respiratory response was even less marked than this, though still perceptible. Thus in 61 per cent of these unselected animals the major portion of the hyperpnea produced by anoxemia, α-lobeline, or cyanide, arose from the carotid chemoreceptors. In 14 of the 49 dogs (28 per cent) the respiratory response was approximately half as great after carotid denervation as before, indicating that the carotid and aortic areas contributed about equal shares to the total respiratory effect. In 5 of the 49 animals (11 per cent) the respiratory response was almost as great after carotid denervation as before; in these exceptional animals the aortic component of the respiratory response evidently exceeded the carotid.

The *circulatory* effects shown in figure 1 are typical of those obtained in 19 out of 36 dogs in which N_2O inhalation was tested before and after aortic denervation. The rise in pressure produced by the inhalation with all nerves intact in these 36 dogs ranged from 18 to 126 and averaged 48 mm. Hg. After inactivation of the vagodepressor nerves the corresponding range was 0 to 76, average 12 mm., this representing the carotid body component. In 19 of these 36 dogs (53 per cent) the carotid component did not exceed 5 mm., in 13 of the 36 (36 per cent) the carotid component ranged from 5 to 30 mm. Hg, and in only 4 of the 36 dogs (11 per cent) did it exceed 30 mm. Thus in our series of unselected dogs, under conditions not far removed from the normal, only exceptional animals showed signs of strong vasomotor reflexes arising from the carotid chemoreceptors in response to acute systemic anoxia. Even in these exceptional cases, the hypertension of carotid body origin was not immediate and sharp, but began 10 to 30 seconds after the onset of the reflex hyperpnea and then progressed gradually; apparently the carotid vasomotor chemoreceptors have a higher threshold than those of the aortic region, or else their influence is too weak to produce hypertension in the face of increasing anoxia of the heart muscle. Certainly in the great majority of our animals the aortic component of the total vasomotor response to systemic anoxia was much more important than the carotid.[3]

[3] This does not mean that reflexes from the carotid bodies are incapable of affecting the circulation reflexly. Evidence has been presented that such reflexes can produce strong reflex bradycardia (Heymans et al., 1933) and reflex vasoconstriction (Heymans et al., 1933, 1935; Bernthal, 1934, 1938). In the present experiments abundant confirmation of these findings was obtained, but the reflex bradycardia (effective through the vagi) was by far the stronger component; it usually overshadowed com-

A diagrammatic representation of the usual parts played by the various chemoreceptor groups in the circulatory and respiratory responses of the dog to acute systemic anoxia is shown in figure 3.

II. *Localization of the aortic chemoreceptors in the dog.* The procedures used for this purpose were the same as those already enumerated (p. 177) with the following additions: Both carotids were denervated at the outset,

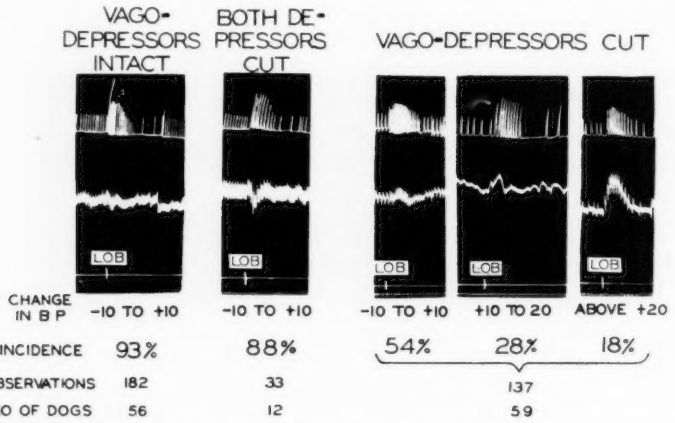

Fig. 2. Vasomotor reflexes from carotid body. Representative tracings of effects produced by intracarotid injections of lobelin (0.1 mgm.). The figures refer to blood pressure changes only; they indicate the number of observations in a large series which were similar to those pictured. Thus, so long as the cardioinhibitory fibres were intact (first two records) no significant change in B.P. occurred in 93 per cent and 88 per cent of the cases. (See footnote[3].) When these nerves were severed (vagodepressors cut) a slight or no increase in B.P. occurred in 54 per cent, a rise of 10–20 mm. Hg occurred in 28 per cent, and an increase of more than 20 mm. Hg in only 18 per cent. A marked hyperpnea was observed in 100 per cent of all 5 groups.

pletely the weaker concomitant stimulation of the vasomotor center (see fig. 2). Comroe and Schmidt (1938) found no reflex hypertension in dogs during perfusion of the carotid body with anoxic blood, but in those animals one or both vagi were intact (although the depressors were cut) and only one carotid body was perfused. In recent experiments (unpublished) on vagotomized dogs with carotid pressure receptors denervated, we have frequently seen reflex hypertension upon perfusion of both carotid bodies with anoxic fluid; injection of cyanide into the fully oxygenated perfusion stream produced some hypertension in 89 per cent of 46 cases, and the average rise in pressure was 34 mm.; in 60 per cent the hypertension exceeded 20 mm. These circumstances are ideal for obtaining maximum reflex hypertension (i.e., perfusion of all functionally active chemoreceptors in an animal with the opposing aortic and carotid pressure receptors denervated and vagi cut). Since no comparable experiment can as yet be done with the aortic chemoreceptors we do not know how greatly the reflex hypertension arising from them would be exaggerated under equivalent circumstances.

so that any reflexes of chemical origin could be attributed to the aortic receptors. A small soft rubber catheter, filled with lobeline or cyanide solution, was passed down a common carotid or subclavian artery, or up the abdominal aorta toward the heart, and with the tip of the catheter at various points lobeline or cyanide was injected through it repeatedly (0.1–0.2 cc. at a time) until the region of maximum reactivity was ascertained. At this point the response was similar to that elicited by intravenous injections of lobeline or cyanide after carotid denervation but the hyperpnea and hypertension following intra-arterial injection appeared

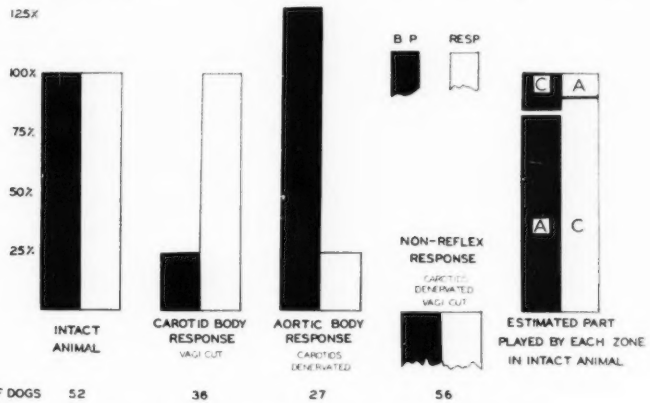

Fig. 3. Schema showing rôles of aortic and carotid bodies in respiratory and circulatory response of the dog to anoxia. If the increase in respiration and B.P. due to anoxia is regarded as 100 per cent for the intact dog, the *carotid* bodies alone are usually capable of producing *respiratory* stimulation equal to that of the entire dog (100 per cent), while the *aortic* area alone usually produces a rise *in B.P.* even greater than that in the intact animal (due to removal of carotid body reflex bradycardia and carotid sinus inhibition by complete carotid denervation). The last column is an estimate of the part played by carotid reflexes (C) and aortic reflexes (A) when both are functioning simultaneously.

immediately and were more intense (fig. 4A). After such a reaction was obtained the catheter was withdrawn measured distances until the response disappeared. In most animals an opening was made in the superior mediastinum and through this the position of the tip of the catheter could be palpated during the experiment; in all cases the position was checked at autopsy.

Experiments of this sort were performed on 38 dogs. Figure 4A shows the positions of the catheters at which positive responses were obtained in each of 33 dogs, while figure 4B shows the positions at which no responses could be obtained, with only 5 exceptions, in the 38 animals. Excepting

these 5, in which some receptors apparently were supplied from the brachiocephalic trunk, the chemosensitive area could always be localized in the ascending aorta or in the first portion of the aortic arch. Confirmation of this finding was obtained in another way, as shown in figure 5.

a. *The blood supply of the aortic chemoreceptors.* At postmortem examination a small vessel was regularly found to arise from the dorsocaudal aspect of the aorta at the level of the brachiocephalic orifice, which corresponds to the region of maximum sensitivity (fig. 4). In only 2 out of 35 dogs examined was this vessel lacking, and in these it arose from the first portion of the brachiocephalic; its subsequent course and distribution were the same in all the animals.

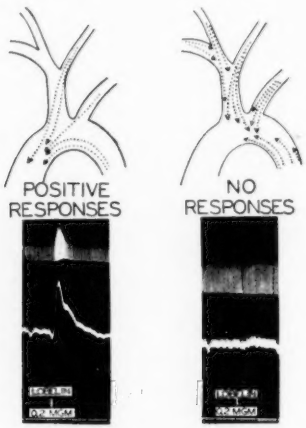

Fig. 4. Localization of aortic chemoreceptors in dog. After carotid denervation fine catheters were passed down a common carotid or subclavian artery or up the abdominal aorta toward the heart. Lobelin or NaCN was injected at various points. The positions at which positive responses were obtained are shown on the left; the positions at which no responses were elicited are shown on the right. Near point of maximum sensitivity there is present regularly a small artery to the aortic body.

This portion of the aorta was submitted to serial sectioning in 8 dogs, and it was regularly found (Addison and Comroe, 1938) that a large cell mass lay in the aortic adventitia surrounding the numerous small branches of this vessel, less than a millimeter from its aortic orifice. This cell mass corresponds closely with the paraganglion aorticum supracardiale of Penitschka (1931) or the aortic body of Nonidez (1937). The structure of the aortic body is very similar to that of the carotid body, both being essentially vascular in nature and containing many nerve endings in intimate relationship with blood spaces (fig. 6).

Nonidez (1937) described other smaller cell groups, lying in newborn

dogs at the very origin of the aorta, which receive their blood supply from a branch of the left coronary artery. We have not as yet been able to confirm the presence of these cell groups in full-grown dogs, though in a few experiments we found that aortic injections produced no response until the catheter was pushed almost to the aortic valves.

Since the aortic body lies between the aorta and the pulmonary artery there is reason to suspect that the receptors may receive blood from the pulmonary artery. This possibility was investigated in 3 dogs by passing

Fig. 5. Localization of aortic chemoreceptors in dog. After injection of lobeline at the mouth of the brachiocephalic had produced no response (A), the catheter was pushed into the ascending aorta (B) and injection of lobelin at this site produced marked hypertension and hyperpnea. In C, although the left subclavian was ligated at its origin and the aorta occluded by inflation of a strong rubber balloon pushed up the abdominal aorta, lobelin produced an immediate and even more pronounced hyperpnea (catheter in same position as in B). The hypertension (C) of course was decreased because of the huge reduction in the vascular bed. Since the brachiocephalic and its branches had previously been found to be insensitive (A), the chemoreceptors or their afferent artery were necessarily located in the ascending aorta (see fig. 4).

one catheter down the right jugular vein into the right ventricle, another up the abdominal aorta into the left ventricle, and injecting lobeline or cyanide through these catheters. The results showed clearly that the receptors do not receive blood from the pulmonary artery, for the responses (hyperpnea and hypertension) from right ventricular injections were delayed 12 to 13 seconds while left ventricular injections were effective in 3 to 4 seconds. Furthermore, examination of the pulmonary artery and its two main subdivisions in 30 consecutive dogs revealed no branches visible with a hand lens.

b. *The nerve supply of the aortic chemoreceptors.* In dogs whose carotids were previously denervated, section of either vagodepressor nerve in the neck diminished, and section of both nerves usually abolished the circulatory and respiratory responses to N_2O inhalation, lobeline and cyanide. If, however, the vagi were cut below the point of entrance of the recurrent

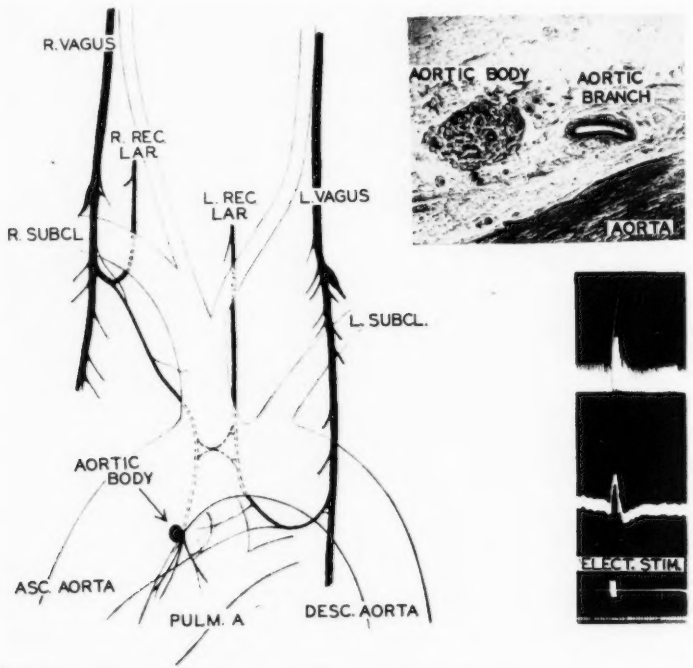

Fig. 6. Location of aortic chemoreceptors in dog. Note the infraaortic position of the aortic body, lying between the aorta and pulmonary artery. Its afferent nerve fibers enter both vagi, probably along with the recurrent laryngeal nerves. Electrical stimulation of the branch to the right vagus produced hyperpnea and hypertension as shown in lower right hand record. Upper right is photomicrograph of the aortic body, showing its afferent artery leaving the aortic wall. The position of this vessel is fairly constant, and coincides with the point of maximum sensitivity to chemical substances (as shown in figs. 4 and 5).

laryngeal nerves but were intact above that point, the responses were not altered in the least. This shows clearly that the afferent pathways for the aortic body reflexes are the aortic or cardiac branches of the vagi. In 4 favorable subjects it was possible to dissect out a nerve running from the aortic body to the right vagus (entering the latter along with the right recurrent laryngeal nerve, fig. 6) and to stimulate it electrically in a spon-

taneously breathing animal: the result was the same as that of an intra-aortic injection of lobeline or cyanide, i.e., intense hyperpnea and distinct hypertension (fig. 6). On the left side we have been unable to identify the corresponding nerve, but believe that it runs with the left recurrent laryngeal because section of the vagus below this level does not alter the systemic or intra-aortic response and stimulation of all branches of the left vagus accessible above the aorta has not as yet reproduced the hyperpneic-hypertensive response.

In one experiment on a dog with denervated carotids it was possible, by crushing the nerves in the vicinity of the aortic body, to abolish completely the aortic chemical reflexes without inactivating the pressure reflexes, as shown by abrupt rise in blood pressure when the vagi were subsequently cut. This observation, together with the occurrence of hyperpnea and hypertension upon stimulating the aortic body nerves directly, indicates that in the aorta, as in the carotids, the pressoreceptors and chemoreceptors are anatomically separable though closely associated.

Chemoreceptors in the cat. The respiratory and circulatory responses of the cat to anoxia, like those of the dog, are dependent upon aortic and carotid reflexes, but the relative parts played by the carotid and aortic bodies are much more variable in the cat. Taking first the *respiratory* response, the carotids appeared to contribute the greater portion of the total hyperpnea of anoxemia and cyanide in 12 out of 26 cats (46 per cent); in 5 of these the aortic response was insignificant. The aortic region was relatively more important in 9 (35 per cent); in 3 of these there was practically no carotid response. In the remaining 5 (19 per cent) each zone appeared to contribute about equally to the total hyperpnea. The average respiratory responses to anoxemia were a 61 per cent increase in minute volume from the carotid bodies alone (average of 23 determinations in 18 cats) and a 64 per cent increase from the aortic region alone (average of 33 inhalations in 20 cats) but the individual variations were much wider in cats than in dogs. It is quite apparent, however, that the aortic body of the cat contributes more to the hyperpnea of anoxemia than that of the dog.

The *circulatory* responses to anoxia in these cats differed from those of dogs similarly prepared in two respects:

First, the hypertension of anoxia was less marked in the cat than in the dog: the average rise in blood pressure produced by 87 N_2O inhalations in 26 cats with all nerves intact was only 22 mm. Hg (range 2–76 mm.), while that in 55 dogs was 40 mm. (range 12–126 mm.).

Second, the partition of this response between the carotid and aortic chemoreceptors was not so clear-cut as in the dog. Of 25 cats, the aortic region was relatively more important to the circulatory response in 11 (44 per cent); in two of these 11, the carotid response was negligible. The carotid region appeared to contribute more than the aortic to the total

circulatory response in 10 (40 per cent); in 3 of these the aortic response was insignificant. In the remaining four (16 per cent) the two zones appeared to contribute about equally. Here again extreme variations were encountered in individual cats, but the *average* anoxemia (N_2O inhalation) increase in blood pressure due to the carotid region alone was 16 mm. (42 determinations in 17 cats), that due to the aortic region alone 15.2 mm. (50 determinations in 22 cats). Similarly, following intravenous NaCN, the average increase in blood pressure due to the carotid bodies was 12 mm. (35 determinations in 16 vagotomized cats); that due to the aortic factor was 13 mm. (36 determinations in 14 cats with carotids denervated).

It is evident that the carotid body is a more important factor in the vasomotor response to acute systemic anoxemia in the cat than in the dog. This was confirmed by intracarotid injections of NaCN, which produced some reflex hypertension in 18 of 23 cats (78 per cent) even though the vagi were intact; in dogs similar injections rarely raised blood pressure as long as these nerves were intact. In only 3 cats however did the rise in blood pressure exceed 15 mm. (maximum 72 mm. Hg in one cat) and the average increase in 91 such injections in the 23 cats was only 9 mm. As in the dog, this was exaggerated by section of the vagodepressor nerves; the average increase in blood pressure following 29 intracarotid injections of cyanide in 14 vagotomized cats was 20 mm. Furthermore some increase in blood pressure was produced by 100 per cent of the injections under these conditions.

Physiological localization of the aortic chemoreceptors was accomplished in 4 cats by passing a fine ureteral catheter filled with NaCN down one common carotid into the left ventricle. In each case the carotids were denervated. Injection of NaCN through the catheter produced immediate hyperpnea and hypertension only when the tip of the catheter was within the left ventricle; the reaction disappeared when the catheter tip lay distal to the aortic valves. This indicates that the blood supply of the aortic body in the cat is derived from the coronary arteries, and this was confirmed by histological studies. Serial sections showed that these physiologically active chemoreceptors in the adult cat lie beneath the aorta near the coronary orifices.

As in the dog, the chemoreceptors do not appear to receive blood from the pulmonary artery, for right ventricular injections (via ureteral catheters passed down an external jugular vein) of cyanide or lobeline regularly showed a considerably longer interval between injection and response than left ventricular injections. The pulmonary artery region was also studied histologically in 6 adult cats: in one a patent vessel arose from the pulmonary artery and entered the chemoreceptor tissue, in another a similar vessel was found but the opening on the pulmonary artery side was obliterated; in the remaining four, no patent vessel or orifice could be found.

The existence of supra-aortic bodies (described by Nonidez in new born kittens) has not yet been confirmed by our preliminary physiological and anatomical studies in adult cats.

As in the dog, the aortic body fibres enter both vagodepressor nerves; section of these invariably abolishes the aortic chemical reflexes in the cat. The course of the fibres from the aortic body to the vagodepressor trunks has not yet been traced.

Discussion. These experiments show clearly that the chemoreceptors first described by Heymans and Heymans (1927) in the cardio-aortic region are localized in the aortic body just as definitely as the carotid chemoreceptors are confined to the carotid body. This is a matter of considerable interest because these two chemoreceptor zones are very similar, not only in function, but in embryological derivation, structure, and relationship to the vascular and nervous systems. Although the localization of the aortic chemoreceptors seems to be accomplished, final conclusions concerning their physiological significance must be postponed. However, several points of value have emerged from the present studies. These will now be considered.

The first point is concerned with the relative physiological importance of the carotid and aortic chemoreceptor areas. There seems to be an unmistakable tendency in the dog for the carotid body to affect the respiratory center more than the vasomotor, and for the aortic body to affect the vasomotor center more than the respiratory. Only the carotid chemoreceptors act directly upon the cardio-inhibitory center. In the cat, the partition of the circulatory and respiratory responses between the two chemoreceptor zones appears in average figures to be much more nearly equal than is the case in the dog, but the averages include wider extremes in the cat.

The second point is the matter of individual variation among different animals of the same species. The experimental conditions under which these observations were made were certainly closer to normal than would have been the case in the perfusion or crossed-circulation experiments that have been used so extensively in investigating these chemoreceptors. In all the present experiments, the chemoreceptor zones and nerves were carefully avoided until we desired to inactivate them. Clearly the weak responses cannot be ascribed to damage to the reflex mechanisms and discarded in favor of the strongest ones, which would then be taken to be representative of the normal state. Instead, the variations must be accepted as inherent in the animals themselves and the factor of individual variability deserves greater prominence than it has been accorded in evaluating results of such experiments. For example, in one dog of this series the aortic respiratory response to anoxemia was far greater than the carotid respiratory effect in many of the animals, yet in only 5 of 49 dogs did the aortic respiratory response exceed the carotid, and in no other

animal did the aortic response closely approach this one in intensity. On the other hand, in 18 of the 49 dogs the aortic respiratory response was barely perceptible. Obviously in a small series one exceptional result of either of these types would incorrectly influence the observer's viewpoint, and conclusions of lasting value can be drawn only from studies on a series of animals sufficiently large to permit determination of the general tendency amid individual variations.

Because of these natural variations individual investigators in this field, having different purposes in view, may arrive at conclusions which seem to be widely divergent but which actually are not at all at variance. An excellent illustration of this is the vasomotor reflex originating in the carotid bodies. If one wishes to demonstrate the maximum influence which this can exert, one may find an occasional dog in which, under suitable conditions, chemical stimulation of the carotid bodies will cause a tremendous rise in blood pressure; in one dog of this series a rise of 138 mm. Hg was observed. Yet this turned out to be distinctly unusual in a large series, and it occurred only after bilateral vagotomy and after selective denervation of the carotid pressure receptors. Heymans and Bouckaert (1939) emphasize the necessity for preventing reduction in the CO_2 tension of the blood (consequent on the simultaneous hyperpnea) by curare and artificial respiration if the carotid body hypertension is to be seen in its fully developed state. Yet if the investigator's purpose is to estimate the importance of this reflex to the intact animal, he cannot base his conclusions on results which are intentionally exaggerated or admittedly exceptional. In the present series it was clear that in the intact anesthetized dog the *carotid body* receptors alone are incapable of stimulating the circulation in times of acute anoxia, despite marked rises in blood pressure obtainable from them in selected animals under highly abnormal conditions. On the other hand the *aortic* chemoreceptors of the dog can and regularly do maintain an increased blood pressure during acute anoxemia, and this despite the opposing influence of the carotid and aortic pressoreceptors, the bradycardia arising from the carotid chemoreceptors, the increasing anoxia of the heart muscle, and the reduction in the CO_2 tension of the blood produced by the concomitant hyperpnea. The carotid chemoreceptors can do this only exceptionally, and then only after the opposing tendencies of aortic pressoreceptors and reflex bradycardia have been eliminated by bilateral vagotomy.

These experiments confirm the belief that the aortic and carotid chemoreceptors together constitute the main and in many cases the sole source of the hyperpnea and hypertension associated with anoxemia and with the action of chemicals such as cyanide; when both sets of receptors are inactivated, systemic anoxia is always much less stimulant than before to respiration and blood pressure; most frequently it is purely depressant

in anesthetized animals. The conclusion that anoxia does not directly stimulate the medullary centers has been fairly generally accepted with regard to respiration, but not to circulation. Our results confirm the conclusions of Brewer (1937) and Lambert and Gellhorn (1938) that the characteristic vasomotor response to anoxia, like the respiratory, is due to reflexes, not to direct central stimulation.

The fact that powerful vasopressor nerve impulses originate in the aortic body and are carried in the vagodepressor nerves is interesting in connection with the reflex described by McDowall (1924). Information obtained from the present experiments indicates that at least a portion of the phenomena described by McDowall originates in the aortic body as a result of chemical changes in the blood. If the vagi are cut when the blood pressure is normal or high, a rise in blood pressure is the rule, for inactivation of the more powerful pressoreceptors occurs simultaneously with inactivation of the chemoreceptors. But if the vagi are cut when blood pressure is *low*, a further fall in blood pressure usually results, for only the chemoreceptors are now inactivated by vagotomy (the pressoreceptors being already partially or wholly inactivated by the subthreshold level of blood pressure). This is in conformity with the observations of Reed (1925) that the McDowall reflex is most consistently observed at the end of long experiments with a low arterial blood pressure. As evidence that removal of vasopressor impulses from aortic chemoreceptors is indeed responsible for the fall in blood pressure when the vagi are cut under such circumstances, an experiment may be cited in which the aortic chemoreceptors of a dog were denervated without severing the vagi or interfering with the innervation of the great veins. In this case, vagotomy led to a rise—and not a fall—in blood pressure even though performed at the end of a long experiment with a low arterial blood pressure.

Furthermore, O_2 inhalation as well as vagotomy may cause an abrupt fall in blood pressure in a cyanotic dog with denervated carotids—an effect presumably due to inactivation of aortic chemoreceptors. It must be pointed out however that oxygen inhalation does not lower pressure as consistently as bilateral vagotomy under similar circumstances, indicating that systemic anoxia is not the only factor in these vagal pressor reflexes. In view of the findings of Bernthal and Weeks (1938) that the carotid chemoreceptors are tonically active under similar experimental conditions, it is probable that the aortic chemoreceptors also set up a continuous discharge of impulses to the vasomotor center. Interruption of this discharge by vagotomy of course would tend to lower blood pressure. In those cases in which vagotomy does, but O_2 inhalation does not lower blood pressure, some other chemical influence, probably increased CO_2, lowered pH, or stagnant anoxia acting through the aortic body, may have been the driving factor. Evidence on this point is not yet available.

SUMMARY

1. The extra-carotid chemoreceptors of the dog have been localized by physiological and anatomical studies in the *aortic body*, a structure fundamentally similar to the carotid body.

2. Both carotid and aortic bodies set up reflexes to the respiratory and vasomotor centers in response to anoxia, whether this be produced systemically by oxygen lack in the inspired air, or locally by interference with tissue oxidations.

3. The major rôle of the aortic chemoreceptors in the dog is the initiation of powerful reflexes to the vasomotor center during anoxemia. By far the greater portion of the hypertension of acute systemic anoxia is produced by aortic body reflexes; vascular reflexes from the carotid body are inconstant and relatively ineffective. The carotid body, however, usually contributes by far the greater portion of the hyperpnea of anoxemia in the dog; the aortic body component, though invariably present, is often insignificant. In the cat the carotid chemoreceptors are relatively more important to the vasomotor response to anoxia than is the case in the dog.

4. The blood supply and afferent nervous pathways for the aortic chemoreceptors have been determined in the dog and cat. In the dog the blood supply is from the transverse aorta, in the cat from the coronary arteries. In both species the nerve fibers reach the vagus trunk close to (probably by way of) the recurrent laryngeal nerves.

5. The possibility that the McDowall reflex may result from chemical stimulation of the aortic body rather than from alterations in venous pressure has been discussed.

6. In view of the close functional and structural similarity to the carotid body, it is proper to use the term *aortic body* suggested by Nonidez, to designate structures now known as *paraganglion aorticum supracardiale, paraganglion of Penitschka, paraganglion aorticum supracardiale superius.*

I wish to express my appreciation to Dr. Carl F. Schmidt for his interest and advice during these experiments and to Dr. W. H. F. Addison for his kindness in preparing the serial sections of the aortic body region.

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ACTION OF PROGESTERONE ON THE GONADOTROPIC ACTIVITY OF THE PITUITARY[1]

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The importance of the ovarian hormones in the regulation of pituitary gonadotropic function has only recently been afforded adequate recognition. It is now generally accepted that estrogen suppresses the follicle stimulating function of the pituitary and there is evidence to show that it also promotes the release of luteinizing hormone in some species such as the rat (Hohlweg, 1934; Fevold, Hisaw and Greep, 1936). Progesterone, however, does not suppress follicle stimulating hormone but it has been shown to inhibit ovulation in rabbits, guinea pigs and rats. Thus, Makepeace, Weinstein and Friedman (1937) showed that ovulation, which normally follows mating in the rabbit, could be prevented if crystalline progesterone were given prior to copulation. These authors concluded that this demonstrated a failure of the pituitary to release gonadotropic hormones in response to the stimulus afforded by copulation since ovulation in these treated animals was readily induced by the injection of gonadotropic material.

Dempsey (1937) likewise showed the progesterone would inhibit preovulatory swelling of follicles, ovulation and corpus luteum formation in the guinea pig, and indicated that this effect was brought about by suppressing the release of luteinizing hormone from the pituitary. Selye, Brown and Collip (1936) found that 4.0 mgm. of progesterone daily would inhibit the estrous cycle of rats and that during the period of injections the ovaries decreased in size and gave no evidence of recent corpus luteum formation. Phillips (1937) has shown that 1.5 mgm. is an adequate daily dose of progesterone for inhibiting the estrous cycle of the rat.

This paper reports experiments which were designed to obtain further information regarding the effect of progesterone on pituitary activity. It is rather generally believed that the release of luteinizing hormone from the pituitary is one essential factor in the induction of pseudopregnancy by cervical stimulation. This reaction was studied to determine whether

[1] Aided by a grant from the Rockefeller Foundation administered by Prof. F. L. Hisaw.

[2] Rockefeller Foundation Fellow in the Natural Sciences.

or not pseudopregnancy could be prevented by progesterone. Also, it is well known that in normal immature rats the injection of follicle stimulating hormone results in luteinization of the ovaries after 5 or 6 days whereas in hypophysectomized rats no luteinization occurs. The luteinization in the intact rats is induced by the release of luteinizing hormone from the animals' own pituitaries under the influence of endogenously produced estrogen (Fevold, Hisaw and Greep, 1936). Studies were therefore made to determine if this secretion of luteinizing hormone could be influenced by progesterone.

EXPERIMENTAL. *Inhibition of pseudopregnancy.* The state of pseudopregnancy in the rat is normally dependent upon the stimulus of copulation during estrus. Greep and Hisaw (1938) have shown, however, that pseudopregnancy may be induced at any time during the estrous cycle by an electric stimulus to the cervix. On the other hand, stimulation during pseudopregnancy, when active corpora lutea were present, was ineffective in prolonging the pseudopregnant period. It was, therefore, suspected that the active corpora lutea in some way interfered with the effectiveness of the stimulus. To test this hypothesis attempts were made to determine if injected progesterone would render the animal refractory to cervical stimulation at times when they normally would respond by becoming pseudopregnant.

Sixty-five normal adult rats, whose estrous cycles were found to be regular, were stimulated cervically with a strong faradic current. Sixty-three of the sixty-five responded to the stimulus by going into the pseudopregnant state. Other groups of similar rats were treated with various doses of progesterone[3] and then stimulated in the same manner either during or immediately after the progesterone treatment.

Figure 1 shows in graphic form the effects obtained in the progesterone-injected animals. The first 4 animals are from the controls and show that pseudopregnancy followed the cervical stimulus after one intervening cycle. Animals 5 to 15 received a total dosage of 1.5 mgm. of progesterone during 3 days, at varying times with respect to cervical stimulation. In all of these animals except one, pseudopregnancy was induced by the stimulus. This amount of progesterone was therefore not sufficient to nullify the effect of the electrical stimulation. Four milligrams were administered to other animals during 3 days (nos. 16–21) and 4 days (nos. 22–26); only 2 of these 11 animals became pseudopregnant, the other 9 continuing to have regular estrous cycles. It is evident, therefore, that this amount of progesterone in some way abolished the normal effect of cervical stimulation.

Inhibition of luteinization. When a purified preparation of follicle

[3] Synthetic progesterone was supplied through the generosity of Dr. Erwin Schwenk of the Schering Corporation.

stimulating hormone is injected into normal immature rats the ovarian response during the first few days is one of pure follicular growth. With

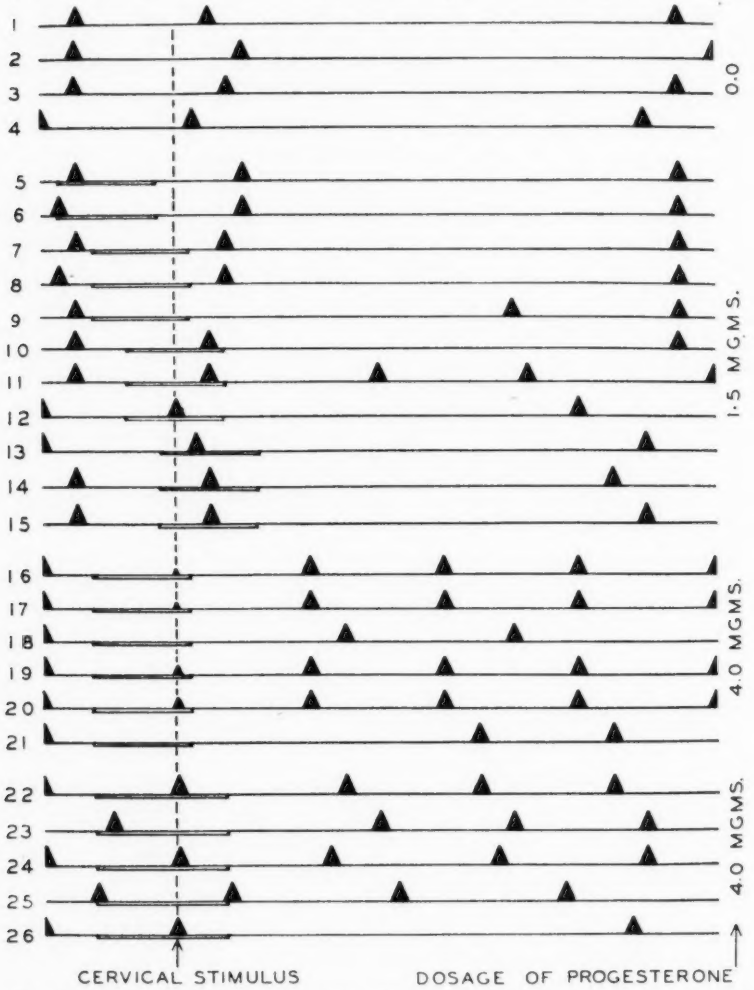

Fig. 1. Showing in diagrammatic form the estrous cycles of rats before and after cervical stimulation. Each triangle indicates the approximate midpoint of estrus; the double line at the left shows the duration of progesterone injections and the total dose is given at the right. It will be noted that 4.0 mgm. of progesterone given during 3 or 4 days inhibited the pseudopregnancy response to cervical stimulation.

continued injections these stimulated follicles become luteinized owing to the participation in the reaction of the animals' own pituitaries. The

degree of luteinization and the time of its first appearance depend in part upon the age of the animal. The older the rats at the beginning of treatment the sooner do they give evidence of the release of luteinizing hormone from the pituitary. Tests were performed to determine the influence of progesterone on this artificially induced pituitary activity.

A standardized follicle stimulating hormone preparation containing only a trace of luteinizing factor and assaying 10 rat units per milligram of dry

TABLE 1

AGE AT START	DAYS TREATED	DAILY DOSE OF PROGESTERONE	NUMBER OF RATS	OVARIAN WEIGHTS	PERCENT LUTEINIZED	
					Partial	Complete*
		mgm.		*mgm.*		
23	4	0.0	4	42.3	25	0
		0.5	2	32.2	0	0
23	7	0.0	4	48.6	75	0
		0.5	2	28.0	0	0
21	7	0.0	6	39.2	17	17
		0.25	6	25.7	0	0
		0.5	5	25.2	0	0
28	7	0.0	4	176.0	100	100
		0.1	5	129.0	80	60
		0.25	5	112.2	100	20
		0.5	5	56.8	20	0
25	10	0.0	3	97.7	67	67
		0.1	5	110.4	100	40
		0.5	5	47.0	60	20
		1.0	3	42.3	0	0
		Hypophysectomized				
29	10	0.0	8	79.6	0	0
		0.5	7	78.0	0	0
		Normal controls				
		0.0	10	130.0	100	90

* Complete luteinization refers to the typical "mulberry" ovaries with many large corpora lutea.

Each animal received 0.25 R.U. of purified follicle stimulating hormone daily in two doses.

powder was used throughout. A fresh solution from this dry preparation was prepared as the tannate on the first day of each experiment and a uniform dose of 0.25 rat unit was given daily in two doses to all groups of animals. The age of the different groups ranged from 21 to 29 days at the beginning of injections, and in each instance controls receiving follicle stimulating hormone alone were treated with those which also received progesterone.

The results of these experiments are shown in table 1. It will be noted that in every case the ovarian response to the injected F.S.H. was inhibited by a daily dose of 0.25 to 1.0 mgm. of progesterone, and in one case 0.1 mgm. showed definite inhibition. The decreased weight in the progesterone treated groups was paralleled by a decrease in the degree of luteinization. A daily dose of 0.5 mgm. completely inhibited luteinization for as long as 10 days in animals 28 days old at the start of treatment. Hypophysectomized rats treated in the same manner showed no luteinization after 10 days of injection, and in these animals 0.5 mgm. progesterone daily exerted no inhibitory influence on the ovarian response. The ovaries of the hypophysectomized rats responded with approximately the same weight increase as did normal animals treated simultaneously with progesterone. The progesterone apparently prevented the pituitaries of the normal animals from participating in the F.S.H. reaction; in other words, it inhibited the release of pituitary L.H., which normally acts synergestically with F.S.H. producing augmentation and luteinization. The fact that luteinization was inhibited and the observation that progesterone depressed the ovarian response to the hypophysectomy level indicates that the mechanism of inhibition in this instance is not mediated by the pituitary release of an inhibitory substance.

Discussion. It seems to be quite clear that in the rat, as in the rabbit and guinea pig, progesterone inhibits the release of luteinizing hormone from the pituitary. It is highly probable that it is by means of this mechanism that the estrous cycle is held in abeyance during periods of corpus luteum activity. This concept carries the implication that luteinizing hormone is a prime motivator in the initiation of cyclic activity and this view is supported by recent work (Fevold, 1939) which shows that estrogen release from the ovary is conditioned by luteinizing hormone.

Although the mechanism whereby cervical stimulation brings about a state of pseudopregnancy is not fully understood, it is probable that the inhibition of this process by progesterone is effected through an inhibition of luteinization. Luteinizing hormone, essential to the initiation of pseudopregnancy, is suppressed by the progesterone and corpus luteum formation is thus prevented.

These observations raise the question of what pituitary factor is responsible for luteal function. In the first place, progesterone injections during established pseudopregnancy or pregnancy do not terminate these states. In other words, progesterone is capable of inhibiting corpus luteum formation without affecting established luteal activity. It is also apparent that the release of luteinizing hormone is held in abeyance at the very times when corpus luteum activity is at its height. During pseudopregnancy, pregnancy and lactation in the rat, periods of active luteal function, the

growth of ovarian follicles is not impeded (Swezy, 1933); these follicles do not undergo preovulatory swelling, ovulation or luteinization but may be made to do so by the injection of luteinizing preparations. Finally, it has been shown by Greep that luteinizing hormone is a potent agent in destroying formed corpora lutea. We are led to the conclusion therefore that the luteinizing hormone is held in check during periods of luteal function and that its eventual release plays a part in the reinitiation of cyclic activity. The pituitary factor responsible for the maintenance of luteal function is probably distinct from luteinizing hormone but as yet the identity of this corpus luteum activator has not been established. Lahr and Riddle (1936) were able to inhibit the estrous cycles of rats with preparations of prolactin, and Nathanson and Fevold (1938) accomplished this result in mice with luteinizing hormone preparations free of lactogenic and adrenotropic activity. In neither of these experiments was it determined whether the inhibition of cycles was brought about by an activation of formed corpora lutea or through a premature luteinization of developing follicles. It would seem that the cycle may be inhibited by either of these mechanisms. Evans, Simpson and Turpienen (1938) have shown that the factor responsible for luteal function is contained in adrenotropic and lactogenic preparations. These authors were able to induce deciduoma formation in hypophysectomized rats treated with these pituitary extracts.

SUMMARY

The injection of 4 mgm. of progesterone during 3 or 4 days into normal adult rats effectively prevented the induction of pseudopregnancy from cervical stimulation. One-tenth to 1 mgm. progesterone injected into immature rats together with follicle stimulation hormone for 4 to 10 days decreased the ovarian weight response and inhibited luteinization. In hypophysectomized immature rats no inhibition was obtained. It is concluded that progesterone suppresses the release of luteinizing hormone from the pituitary and that this suppression is responsible for the absence of estrous cycles during periods of active luteal function. Some pituitary hormone other than luteinizing hormone is responsible for the continued function of formed corpora lutea.

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FURTHER OBSERVATIONS ON THE CARBOHYDRATE, FAT, AND PROTEIN APPETITE OF VITAMIN B DEFICIENT RATS

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Previous self-selection experiments with purified substances showed that rats on a diet deficient in the vitamin B complex had an increased appetite for fat and an aversion, or at least a greatly reduced appetite, for carbohydrate and protein (Richter, Holt, Barelare, and Hawkes, 1938). The appetite for these substances returned to normal when the rats were given access to foods with a high vitamin B content, such as yeast, or liver powder. Since, in general, the rats' dietary selections have a close relationship to their internal needs, it was concluded from these experiments that vitamin B deficient rats are unable to use carbohydrate and protein, but can utilize fat. These results agreed with the findings of Evans and Lepkovsky (1929), who reported that fat "spares" vitamin B, and with the findings of various workers (Peters and Thompson, 1934; Thompson and Johnson, 1935; Platt and Lu, 1936), who have shown that vitamin B deficient animals suffer some derangement in their carbohydrate metabolism, as evidenced by the increased amounts of pyruvate (bisulphite-binding substances) in the blood.

Since the diet of these animals lacked all of the components of the B complex—thiamin, riboflavin, nicotinic acid, W factor, B_6, and undoubtedly other factors—the changes in fat, protein, and carbohydrate appetite had to be regarded as the composite effect of the absence of all these components. Further experiments have now been undertaken to establish the specific relation of each of these components to the appetite changes. In view of the fact that carbohydrate utilization depends largely on the presence of thiamin, we wanted to know especially whether rats, when given access to thiamin and none of the other components of the B complex, would indicate by their appetite an ability to use carbohydrate. Similarly, we wanted to know what changes in appetite would be produced by the ingestion of riboflavin, nicotinic acid, and W factor when offered singly and in various combinations with thiamin.

METHODS. The cages used in these experiments consisted of a revolving drum, a cyclometer, and a living compartment (Richter, Holt, and Bare-

lare, 1938). The living compartment contained three food cups for solid substances and eight graduated inverted drinking fountains for fluid substances. The living compartment and the drum were made of a wire cloth with a mesh sufficiently large to let the feces drop through freely.

Daily readings were made of activity, food and water intake, and vaginal smears. The animals were weighed weekly. These 4 readings and the readings of the intake of each of the 11 substances were plotted one above the other on a separate chart for each animal.

In these experiments, as in the earlier experiments, we placed the rats in activity cages at an age of 45 to 50 days, kept them on the standard McCollum diet for approximately 18 days, and then offered them the self-selection diet.

On the self-selection diet the rats had access to 11 substances in separate containers, one representative of most all of the substances known to play an important part in normal nutrition, and water. Fat was represented by olive oil; protein, by casein (autoclaved and purified); carbohydrate, by sucrose; and the minerals, by sodium chloride, aqueous solution 3 per cent, dibasic sodium phosphate 8 per cent, magnesium chloride 0.5 per cent, potassium chloride 1 per cent, calcium lactate 2.4 per cent; the vitamins A and D, by cod liver oil; and vitamin B, by dried baker's yeast.

From previous experiments we know that on the selections made from this assortment of substances, rats show normal growth, activity, and reproduction (Richter, Holt, and Barelare, 1938).

The self-selection technique greatly simplifies the production of a dietary deficiency. As each of the substances is offered in a separate container and in purified, or nearly purified, form, a deficiency can be produced simply by removing one container. Thus, in the present experiments vitamin B deficiency was produced by omitting the yeast from the diet, since none of the remaining substances contained any vitamin B.

These experiments were carried out in two series. In the first, or control series, we made observations on the appetite of rats which received: 1, the full self-selection diet; 2, the self-selection diet minus yeast; and 3, the self-selection diet minus yeast and fat. In the second, or experimental, series, we studied changes in appetite of animals offered the self-selection diet from which yeast was omitted and one of the vitamin B components—thiamin, riboflavin, nicotinic acid, or W factor—or a combination of two or four of these factors, was made accessible.[1]

Forty-nine females were used in the experiments. At least four animals

[1] Merck & Co., Inc., generously supplied the riboflavin and a large part of the thiamin used in these experiments. E. R. Squibb & Sons kindly supplied some of the thiamin. The W factor concentrate was prepared by Dr. D. V. Frost and sent to us in very liberal amounts by Dr. Conrad Elvehjem.

were used in each one of the experiments with the choice of single vitamin B components and of the combinations.

All of the animals were autopsied at death. The glands of internal secretion were weighed and preserved for histological study.

RESULTS. *Full self-selection diet.* At an average age of 50 days, 12 rats which previously had received the standard McCollum diet were placed on the full self-selection diet. They showed normal growth, activity, and regular 4 to 5 day oestrous cycles. After 50 days on the diet their daily caloric intake averaged 46 calories, approximately 18 per cent less than the average intake of 56 calories of control rats of the same age on the standard McCollum diet. Figure 1A (solid line) shows the average carbohydrate, fat, and protein intake of the 12 rats for the first 70 days on the self-selection diet. The carbohydrate intake (sucrose plus carbohydrate in yeast) averaged approximately 6 grams per day; fat intake (olive oil), 2 cc.; and protein (casein plus protein in yeast), slightly more than 2 grams. The intake of each of these three food factors remained fairly constant throughout the 70 days.

Ten of the 12 rats gave curves which resembled the average curves presented in figure 1A. The 2 exceptions were rats which ate more fat than carbohydrate. All 12 rats gave very similar protein intake curves.

Self-selection diet without yeast (vitamin B). In this group we kept 5 rats under identically the same conditions as the full diet group, except that we did not give them access to yeast. They started to lose weight at once and continued to do so at a very constant rate until they died. The survival period averaged 65 days. Cornified cells disappeared from the vaginal smears after 13 to 23 days.

In agreement with our previous observations these animals showed very definite changes in appetite. They reduced their sucrose intake markedly, stopped eating casein almost entirely, but sharply increased their olive oil intake. Figure 1A gives the average intake curves for the 5 animals in the group (broken line). The sucrose intake decreased from an average of 5.3 grams for the first 10 days to an average of 1.3 grams during the 60 to 70 day period. For the first 30 days the olive oil intake surpassed the normal average; after that it decreased slowly to a lower level. Casein intake decreased in 20 days from an average of 1.8 grams to a level of 0.2 gram.

All 5 rats developed an almost complete aversion to casein. The daily intake rarely averaged more than 0.2 gram after the first 30 days on the diet. Four out of the 5 rats showed a very low sucrose and a high olive oil intake; 1 animal which lived only 51 days ate no olive oil and only a small amount of sucrose. In our previous study all 9 rats showed a decreased sucrose intake and an aversion to casein; 8 showed an increased olive oil intake.

Thus, these vitamin B deficient rats showed a preference for fat and an aversion to carbohydrate and protein.

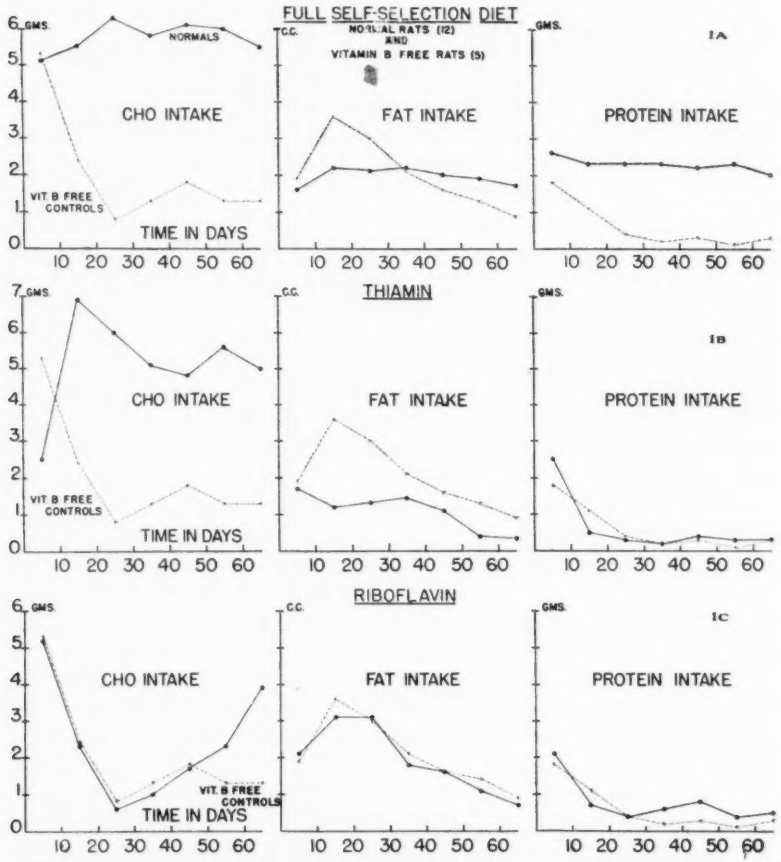

Fig. 1A. Average daily intake of carbohydrate, fat, and protein in 10 day averages of (solid line) 12 rats on the full self-selection diet and (broken line) 5 rats on the self-selection diet without yeast for the first 70 days on the self-selection diet.

Fig. 1B. Average daily intake of carbohydrate, fat, and protein of (solid line) 4 rats offered thiamin as compared to that of the group on the yeast free diet.

Fig. 1C. Average daily intake of carbohydrate, fat, and protein of (solid line) 4 rats offered riboflavin as compared to that of the group on the yeast free diet.

Self-selection diet without yeast (vitamin B) and fat. Further experiments with 6 rats were undertaken to determine whether by omitting fat from their diet we could force them to eat either carbohydrate or protein;

and if so, whether they would fare better or worse than the animals on the diet containing fat. They ingested fair amounts of sucrose, taking an average of 7.1 grams during the first 10 days, decreasing gradually to 2.0 grams during the 60 to 70 day period, but still refused almost completely to eat casein. They had a greater aversion to protein than to carbohydrate. All 6 rats showed similar curves.

That the choice of a high fat diet actually benefited vitamin B deficient animals is apparent from the fact that these vitamin B deficient rats on the no-fat diet survived only 51 days, 14 days less than the yeast free controls, which had access to olive oil.

Components of the vitamin B complex: Daily intake. In agreement with our previous observations we found that the rats drank the thiamin and riboflavin solutions freely (Richter, Holt, and Barelare, 1937). The solutions were offered in concentrations which were determined on a purely empirical basis. We tried to adjust the concentrations so that, when we made the solutions accessible to the rats, the daily intake would average between 1 and 5 cc. for most animals. We deducted 0.8 cc. per day for evaporation from all aqueous solution readings. The intake of thiamin (aqueous solution 20 mgm. per 100 cc.) averaged 3.5 cc., or 0.7 mgm., per day for 70 days on the self-selection diet. This is much more thiamin than is required daily by the rat. The intake of riboflavin (aqueous solution 2 mgm. per 100 cc.) averaged 2.5 cc., or 0.05 mgm., per day for the same period. The intake of nicotinic acid solution (aqueous solution 500 mgm. per 100 cc.) averaged 0.3 cc., or 1.5 mgm., per day. Since the amounts taken daily of the nicotinic acid solution were so small, scarcely larger than the reading errors and variations due to evaporation, these figures are only approximations. A more dilute solution of nicotinic acid would probably have given truer results, but we were misled by a few preliminary trials in which some animals drank larger amounts of the concentration used here. The W factor was offered in concentration of 1 cc. of the extract to 25 cc. of water. The daily intake averaged 3.4 cc., or 0.13 cc. of the extract. The intake of this substance varied greatly from animal to animal and also in the same animal at different times. Some animals drank as much as 35 cc. of the solution per day on several occasions.

Survival periods. The animals which received any one of the vitamin B factors lived longer than the yeast free (vitamin B free) controls. The riboflavin rats lived longest, 106 days; the thiamin rats lived 95 days; the nicotinic acid rats, 85 days, while the yeast free controls lived 65 days and the yeast and fat free controls only 51 days. The survival times were fairly consistent within each group, except in the W factor group. We are unable to give an average survival time for the 6 W factor rats. Three died after an average of 72 days; two were killed after 60 days, and one

after 129 days. This last animal gave all indication of being capable of living for a much longer time.

Body weight. The rats which had access to the single vitamin B components lost weight at practically the same rate as the control rats on the vitamin B free diet. None of the vitamin B components, when offered singly, increased, or even maintained, body weight.

Activity. All of the rats, even those on the yeast (or vitamin B) free diet, showed a surprising amount of activity. They remained active usually until within several weeks before death. The thiamin rats showed the greatest activity; the riboflavin rats, the lowest.

Reproductive tract. The vaginal smears of the rats on the self-selection diet without yeast, but with thiamin or with nicotinic acid, were similar to those found in the yeast free control rats. The cycles became irregular almost at once and disappeared after 20 days leaving a constant dioestrous picture.

The 4 rats on riboflavin also showed irregular smears for the first 20 days on the self-selection diet with a subsequent disappearance of the cycles. However, about the 40th day they began showing cyclical changes again, on the whole rather irregular, but in two instances quite as regular as were shown by the rats on the full self-selection diet. These cycles disappeared again after 70 to 90 days on the diet.

The 6 rats on W factor also gave smears which indicated some, though slight, replacement in the reproductive tract. Five of the rats showed a few sporadic days of cornification after 30 days on the diet; the rat which was still alive and doing well after 129 days showed irregular cycles of cornified cells for the period between 90-110 days.

Carbohydrate, fat, and protein appetite: Thiamin. The appetite of the 4 rats on the self-selection diet without yeast, but with thiamin, contrasted sharply with that of the yeast free controls. Whereas the latter animals refused sucrose and ate large amounts of fat, all 4 of these rats ate large amounts of sucrose and small amounts of olive oil. Like the controls, however, they refused almost entirely to eat casein. For the greater part of the time these rats lived almost exclusively on sucrose.

In figure 1B it will be seen that the daily sucrose intake rose sharply from an average level of 2.5 grams during the first 10 days on the diet to 6.9 grams. For the remaining 50 days it averaged slightly more than 5 grams per day. The olive oil intake fell from an average daily intake of 1.7 cc. to about 1.3 cc. for the next 40 days, after which it dropped to about 0.3 cc. daily. The casein intake was practically the same as that of the vitamin B free controls and, after the first 20 days on the diet, averaged 0.3 gram per day.

Riboflavin. The sucrose and olive oil appetite of the riboflavin rats more closely approximated that of the yeast free controls; their casein

appetite, however, was definitely increased. (See fig. 1C.) All 4 rats showed similar sucrose and olive oil intake curves; 3 out of the 4 showed the increased casein appetite.

Nicotinic acid. The appetite of the nicotinic acid rats closely resembled that of the yeast free controls. The sucrose intake decreased sharply from a daily average of 4.4 grams during the first 10 days to a level of 0.8 gram per day, then rose to 2.5 grams per day during the 60 to 70 day period. The olive oil intake was quite low, 1.0 cc. daily for the first 10 days, then rose to an average of 2.3 cc. for the next 40 days, with a subsequent decrease to 0.6 cc. during the last 10 day period. The casein intake was quite normal at the beginning, 2.3 grams, but fell rapidly to an average of about 0.2 gram for the remainder of the test period.

In view of the fact that these animals chose carbohydrate, fat, and protein in about the same amounts as the yeast free controls, it would appear that their longer survival was due to factors not directly concerned with the fat, carbohydrate, and protein metabolism.

W factor. The appetite of the 6 rats on W factor differed from that of both the yeast free and thiamin rats. With one exception these rats ingested less sucrose than the yeast free controls; in fact, they showed almost an aversion to sucrose. Five of the 6 rats ate large amounts of olive oil. Their daily olive oil intake curve very closely paralleled the normal curve, but was slightly higher and remained constant during the 70 day period. Five out of the 6 animals showed a very low casein intake, even lower than that of the yeast free controls. The remaining animal, however, which was still alive and active after 129 days on the diet ate some casein at all times, definitely more than was eaten by any of the vitamin B free controls. This animal ingested very large quantities of W factor solution.

The maintenance of the fat intake at a high and constant level throughout the 70 day test period may indicate a close dependence of fat metabolism on some ingredient contained in the W factor extract or may be due simply to the lack of thiamin.

Combination of vitamin B factors: Thiamin and riboflavin. In these experiments 4 rats on the self-selection diet without yeast had access to thiamin and riboflavin offered in separate containers and in the same concentrations as in the single factor experiments.

The thiamin intake averaged 2.1 cc., or 0.42 mgm., per day, slightly more than one-half as much as taken by the group on thiamin alone. The riboflavin intake averaged almost the same as that of the group on riboflavin alone, 2.0 cc., or 0.04 mgm., per day. As in the single factor experiments, the intake of these substances was quite constant throughout the 70 day period.

Two of the 4 animals died after 53 and 94 days respectively, but the other two gave all indications of living indefinitely and were killed after 165

days on the diet. For the first 50 days all of the animals lost weight at much the same rate, slightly less rapidly than the vitamin B controls. After that the two animals which survived stopped losing weight and even started to gain weight at a moderate rate. One of these animals was still gaining weight at the end of 165 days when it was killed. The activity of these animals did not deviate very far from the normal average. The vaginal smears of the two shortlived animals became dioestrous at about the same time as the yeast free controls; in one of the longlived animals

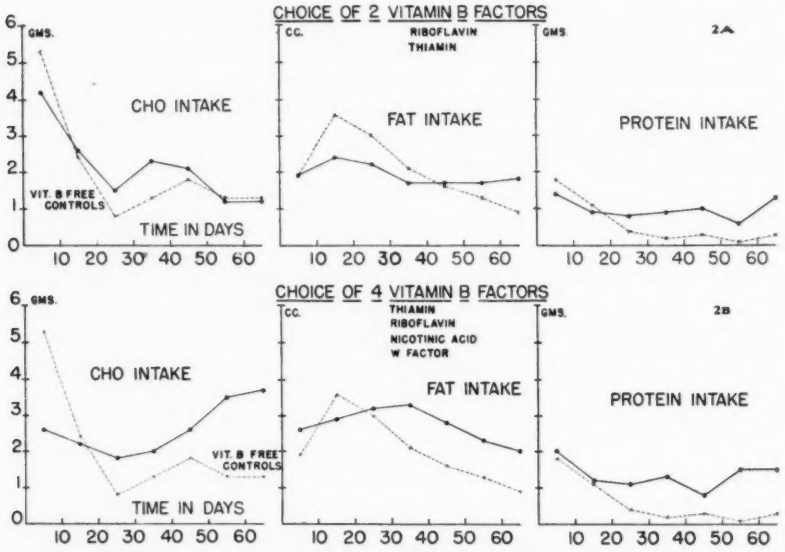

Fig. 2A. Average daily intake of carbohydrate, fat, and protein of (solid line) 4 rats in the two-combination group as compared to that of the group on the yeast free diet.

Fig. 2B. Average daily intake of carbohydrate, fat, and protein of (solid line) 4 rats in the four-combination group as compared to that of the group on the yeast free diet.

cornified cells appeared at fairly regular intervals of 8 to 12 days as long as 167 days after the diet was started. The other longlived animal showed a dioestrous smear from the start of the experimental diet with only occasional days of cornification.

Carbohydrate, fat, and protein appetite. In general, the carbohydrate, fat, and protein appetite of these animals, especially of the two which survived, changed in the direction toward the normal. Sucrose intake showed a small increase over the yeast free level; olive oil, a slight decrease; and casein showed a marked increase. (See fig. 2A.) During the last 50 days

of observation the daily casein intake averaged 1.0 gram, as compared to 0.2 gram of the yeast free control rats.

Clearly, the thiamin and riboflavin had effects which supplemented one another. On the basis of the results of the single factor experiments, it would appear that the thiamin had enabled the rats to utilize more sucrose and thus to eat more of it. In the same way, the riboflavin appeared to allow greater utilization and greater consumption of casein.

Thiamin, riboflavin, nicotinic acid, and W factor. In these experiments the rats had access to the four vitamin B factors, all given in the same concentrations used in the single factor experiments.

The thiamin intake averaged 0.4 cc., or 0.08 mgm., per day, which is only about one-fifth the amount taken by the group on thiamin and riboflavin and about one-ninth the amount taken by the group on thiamin alone. The riboflavin intake averaged 2.5 cc., or 0.05 mgm., per day, which is the same as that taken by the group on riboflavin alone and only slightly greater than that taken by the group on thiamin and riboflavin. The nicotinic acid intake averaged 0.17 cc., or 0.9 mgm., per day, which is slightly more than one-half as much as that taken by the group on nicotinic acid alone. The W factor intake averaged 2.8 cc., or 0.06 cc. of crude extract, only slightly less than that taken by the group on W factor alone.

With the combination of the four factors these animals throve better than animals on any of the other selections. At the end of 109 days, when they were autopsied, they were still gaining weight and gave all indications of living indefinitely. The body weight curve of one animal approximated the average body weight curve of the 12 rats on the full self-selection diet. They were not as active as the rats on the single factors, but their activity fell within the normal limits of variation. Their vaginal smears showed frequent, though usually irregular, days of cornification. One animal had quite regular 4 to 5 day cycles.

Carbohydrate, fat, and protein appetite. The carbohydrate, fat, and protein appetites of these animals tended to resemble the appetite of rats on the full self-selection diet. (See fig. 2B.) However, they still fell short of a complete duplication. The daily sucrose intake during the first 10 days was 2.6 grams and increased to 3.6 grams after 50 days on the diet; the olive oil intake averaged 3.0 cc. daily for the first 40 days, then decreased gradually to 2.0 cc. The casein appetite showed a marked change, averaging 1.3 grams throughout the 70 day period. This represented a very significant increase over that of the animals on any of the single components or the combination of 2 components.

In view of the better growth and survival in the four-combination group, as compared to the two-combination group, it appeared that the ingestion of nicotinic acid and of the W factor extract helped to supplement beneficial effects from the thiamin and riboflavin.

Mineral appetite. With the self-selection technique we obtained separate records also of the intake of 5 different mineral solutions—sodium chloride, dibasic sodium phosphate, potassium chloride, magnesium chloride, and calcium lactate. With the development of vitamin B deficiency the intake of each of these mineral solutions decreased. Only the combination of the four vitamin B factors sufficed to maintain the intake of any of these minerals near the normal level.

Discussion. In view of the great consistency of the protein appetite in rats on a normal diet, we must place considerable emphasis on the striking changes in protein appetite that occurred in the rats on a partial or complete vitamin B free diet. The rats deprived of all vitamin B showed an aversion to protein. For the most part they rarely ingested any casein and could be forced to partake of it only with great difficulty, even when other substances were withheld. The close interrelationship between the degree of maintenance and the appetite for protein brings further evidence for the importance of the rôle played by protein in vitamin B deficiency.

Of the four vitamin B components tested so far riboflavin had the most pronounced effect on casein appetite. However, we must not overlook the striking effect produced by W factor in one animal. Also, the protein appetite may be influenced by other factors, such as iron, which plays an important rôle with riboflavin and protein in a cellular oxidation-reduction system (Warburg's yellow enzyme).

Of interest is the fact that with riboflavin the rats not only ate more protein, but also lived longer than the rats on any of the other components and showed a much greater maintenance effect on the reproductive tract.

The changes in carbohydrate appetite shown by these animals agree with our knowledge of the part played by thiamin in the carbohydrate metabolism (Evans and Lepkovsky, 1929). Without any components of the B complex the rats ate only small amounts of sucrose. However, when given thiamin and none of the other components of the B complex, they all ate sucrose and stopped taking olive oil almost entirely. Since addition of any of the other factors did not bring out a carbohydrate appetite, it would appear that the carbohydrate appetite depends on the presence of thiamin.

It is noteworthy that rats which receive thiamin and none of the other components of the B complex should be almost exclusively "sugar burners." The strong and almost exclusive sucrose craving occasionally seen in humans may have a similar origin.

The factors involved in the regulation of the fat appetite are less clear. Since, in general, fat and sucrose appetite vary inversely, the increased fat appetite of rats deficient in all of the components of the vitamin B complex may have its origin in the decreased sucrose appetite. It has been reported, however, that vitamin B deficient rats thrive better on a

high fat diet, due to the supposed vitamin B sparing action of fat. The only indication that we found of a direct relationship between the fat appetite and one of the B factors came from the W factor experiments. Most of the rats which received only the W factor showed a greater and more sustained fat appetite than any of the other animals. In one W factor animal also the protein appetite was increased. It may be that this variety of effects has its origin in other components still present in the W factor extract.

The other components of the B complex, B_6, etc., as well as the other ingredients of yeast, such as nucleic acid and invertase, may, of course, also play an important part in the regulation of the appetite for fat, protein, and carbohydrate.

SUMMARY

1. Rats which previously had access to the standard McCollum diet were offered, at the age of 65 days, a self-selection diet which contained no part of the vitamin B complex.

2. These animals ingested more olive oil, much less sucrose, and practically no casein, as compared to control rats which received a self-selection diet containing yeast.

3. Six groups of rats on the basal vitamin B free self-selection diet were given access to one or several of the following vitamin B components: thiamin, riboflavin, nicotinic acid, and W factor. The rats showed an appetite for each of these components.

4. On riboflavin the survival time averaged 106 days; on thiamin, 95 days; on nicotinic acid, 85 days. When entirely deprived of all vitamin B, survival time averaged only 65 days.

5. Riboflavin had more effect than any of the other components on the reproductive tract.

6. The group of rats which was given access to thiamin in addition to the basal vitamin B free self-selection diet ate normal amounts of sucrose, stopped drinking olive oil, but refused to eat casein.

7. The group given access to riboflavin displayed an increased appetite for casein.

8. The rats given access to nicotinic acid had much the same appetite as the vitamin B free controls.

9. The rats offered W factor showed a constant and normal olive oil intake.

10. The rats which had access to thiamin and riboflavin, or all four components, tended to show an appetite for sucrose and olive oil similar to that of the control animals offered the self-selection diet containing yeast; but the most striking effect brought about by these combinations of vitamin B components was the return of the casein appetite.

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